THE BEHAVIORAL PHYSIOLOGY OF COMPETITIVE ABILITY IN RECENTLY WILD-DERIVED MALE HOUSE MICE (MUS MUSCULUS)

by

Christopher B. Cunningham

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The dissertation of **Christopher B. Cunningham**

has been approved by the following supervisory committee members:

David R. Carrier	, Chair	**12/14/2011** Date Approved
Fredrick R. Adler	, Member	**12/14/2011** Date Approved
Elisabeth A. Cashdan	, Member	**12/04/2011** Date Approved
Jon A. Seger	, Member	**12/14/2011** Date Approved
Wayne K. Potts	, Member	**12/14/2011** Date Approved

and by **Neil J. Vickers** , Chair of the Department of **Biology**

and by Donna M. White, Interim Dean of The Graduate School.

ABSTRACT

Social dominance is the most important known behavior to reproductive success of males across the animal kingdom. A high social dominance rank is usually gained by physical competition or signals displaying competitor quality. Despite its importance in shaping the diversity seen in the animal kingdom, little is known about specific traits that promote high competitive ability within an individual. In this dissertation, I begin to elucidate some of the behavioral physiology underpinning competitive ability in the premier mammalian model system, the house mice (*Mus musculus*). House mice are ideally suited for this study because of a well-characterized natural history demonstrating that dominant males gain ~90% of all fitness, while still having to perform many other behaviors, such as foraging.

In this dissertation, I provide an overview of the some hypothesized constraints on the evolution of competitive ability and phenotypic trade-offs with other important life-history traits. Second, I describe an experiment that investigated multiple traits at several levels of biological for their possible influence on competitive ability. I demonstrate that competitive ability is heritable, moderately influenced by relative body mass, and negatively influence by litter sex ratio. No effect of litter size, relative age, or placement order was seen. Third, I demonstrate that aggression and competitive ability are distinct phenomena in

this system. Next, I demonstrate that primary signaling pheromone of house mice, major urinary proteins, do not advertise rank but are responsive to social experience. Finally, I switch clades and demonstrate that relative brain size in primates is positively associated with intensity of male-male competition. Collectively, this project demonstrates that competitive ability is an extremely complicated phenotype and merits a great deal more study.

To my family, who suffered through many Utah winters

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CHAPTER 1

SOCIAL DOMINANCE IN MALE HOUSE MICE: A BRIEF SUMMARY AND LIFE-HISTORY TRADEOFFS

1.1 General introduction to social dominance

Physical competition is one of the most intriguing behaviors driving the evolution of many animal species. Within a social species, the outcome of physical agonistic competition leads to ranking with in a hierarchical social network. Restricting the discussion to mammals, it is the single largest known driver of fitness in males of most species. With very few known exceptions, in fact, reaching a high position within a social hierarchy is the only way in which a male can achieve reproduction. This statement is especially true within Carnivora, Rodentia, Ungulata, and Primates [1].

Given the reproductive advantages of occupying a dominant social position, it is surprising that relatively little is known about the behavioral physiology that underpins competitive ability. Studies on characters associated with competitive ability have generally investigated only one or two traits. For example, weapon size has been shown to be positively correlated with breeding success in primates [2] and lizards [3, 4]. Body size has long been thought to influence competitive ability [5, 6], but has been demonstrated to be of little

impact to competitive ability in some species [2, 3]. Locomotor performance is positively correlated with fitness in lizards, specifically, sprint speed [7, 8] and endurance [9]. Although these studies provide evidence for traits that are causally associated with competitive ability, there remain many unstudied traits at many levels of biological organization that likely influence competitive ability. Most importantly, the full suite of characters associated with competitive ability and the extent to which selection for competitive ability influences other aspects of life history are not fully understood for any species.

The following discussion provides a theoretical framework of some major selection pressures that are possibly driving and constraining the evolution of competitive ability in male house mice (*Mus musculus*). It also serves as a brief background of the current project and the life history of the house mouse, which make it an ideal study system for this type of research. I approached this project as though competitive ability is a trade-off phenotype with many other life history traits. This introduction is not meant to provide an exhaustive review of all possible selection pressures on male house mice and is strictly applicable to free-living commensal house mice or close approximations. At the end of this introduction, I hope to provide part of an answer to the question: Why are there subordinate males, i.e., low competitive ability males?

First, it is necessary to define some terms and concepts that will be used in the following discussion. Competitive ability is the capacity of an individual to win physical agonistic encounters. This ability is directly associated with the capacity to gain and hold reproductive resources. Competitive ability is the

competitive ability of an individual within a hierarchical social network of a social species. These abilities are innate traits that do not require a social environment. Alternatively, they can be viewed as the probability that an individual male will win any given physical conflict. Within house mice, this trait is expressed when males gain and hold prime territories. Competitive ability and competitive ability are synonymous terms that will be used interchangeably throughout the rest of this introduction. Competitive is the outcome of physical agonistic encounters in a social environment and is equivalent to social rank and like terms. The difference between “social dominance ability” and “social dominance” can be viewed as the probability that a male will be dominant versus whether the male did actually attain a dominant social position. A trade-off phenotype is a character state that represents an optimization between two or more behaviors that an animal must perform. By definition, a character state that increases the ability of an individual to complete one behavior will make it harder to complete the other behavior(s). For example, individuals who wish to run efficiently generally reduce the amount of muscle mass in the distal portions of the limbs. In contrast, individuals who wish to manipulate effectively an opponent want muscle mass throughout the limb to increase force production. Therefore, it is impossible to be both specialized to run and fight at peak efficacy at the same time. This is a functional tradeoff.

1.2 Social dominance and *Mus* biology

The social rank of a male house mouse nearly completely predicts its lifetime fitness at high population densities [10]. Dominance rank is established by the outcomes of multiple agonistic encounters with conspecifics [11, 12]. Dominance is important to males because populations, regardless of density or stability, appear to be characterized by a polygynous mating system [13, 14]. This physical competition leads to the demic social structure characteristic of house mice with a dominant male defending a territory that usually includes several breeding females, some of their offspring, and perhaps a few subordinate males [13, 15]. When males are placed in laboratory seminatural enclosures, fighting is intense [16, 17] and a clear social hierarchy quickly forms as is seen in natural settings [16-20]. While there are no experiments assessing paternity in wild populations in relation to dominance rank, in experiments using seminatural enclosures, female house mice mate primarily or exclusively with the dominant males [17, 19-21]. For example, studies of the mouse colony used in this experiment have shown that territorial males sire 80% of the progeny, although they represent only 50% of the available male mates. The polygynous mating system and intense physical competition of house mice are highly relevant to most mammalian mating systems [5].

These characters combined with their short reproductive cycle, ability to thrive in seminatural enclosures, relative tolerance of human experimenters, and all the tools associated with a model organism make house mice an ideal species

to perform many experiments with and are the reasons that they were chosen for this experiment.

1.3 Phenotypic tradeoffs with competitive ability

Moving from relevant life history characteristics and into an experimental mindset, there are many hypotheses to be tested about possible tradeoffs and direct questions of how an individual male attains social dominance. Because competitive ability is heritable and is the primary trait that determines fitness in males, it raises the possibility that male phenotypes can be optimized for this behavior. However, it is unlikely that the combination of alleles that promote competitive ability would be driven to fixation due to competing functional tradeoffs with other life history demands. Wild house mice likely experience conflicting selection pressures against competitive ability from many other necessary and important tasks. These might include efficient foraging, periods of low resources, immunocompetence, antagonistic sexual selection, and paternal care. Collectively, all of these ecologically and environmentally relevant selective tradeoffs may increase the population variation in alleles associated with competitive ability.

Physical conflict is not the only evolutionarily relevant behavior of male house mice. House mice are also an extremely successful colonizing species. Additionally, they are a species that forages great distances on a daily basis. These two behaviors should present formidable counter selective pressures on the ability of males to be optimized for competitive ability.

Dispersal is a characteristic of an overwhelming percentage of house mouse populations. Dispersal is also sexually biased towards males. Males approaching reproductive age are usually forced to migrate and colonize new territory by the dominant male of his natal deme [13, 22]. Dispersal is generally thought to be driven by three factors: (a) a high fertility rate; (b) aggression by territorial males and lactating females; and (c) a saturated carrying capacity [14, 22]. Roughly 80% of the young male mice produced each year disperse from their natal population [23]. Dispersal is such an important life history component of house mouse biology that investigations into its mechanistic basis have been completed. There are two MUP isoforms that are expressed at an ontogenetic time point that coincides with male progeny being aggressed upon to leave the natal deme [24].

Mice must also forage daily. Although to date, no measurements of daily travel distances for wild house mice are known, outbred laboratory mice will voluntarily run an average of 4.4 km a day [25]. This is equivalent to the average American male running ~ 90 km a day. It should also be noted that laboratory mice are relatively less active than colonies of recently derived mice. Even if males are constrained in distance they can travel due to neighboring territories, foraging and patrolling territory should still present a reason to minimize the cost of transport.

Both male-biased dispersal and large foraging demands are two life history traits that might that prevent specialization for competitive ability. A population that demonstrates a tradeoff between locomotor efficiency and

competitive ability has been documented in one reptile species. In marine iguanas, the demands of foraging selects for reduced size while their lek mating system, in which males compete physically, selects for increased body [26]. Tradeoffs between locomotion and competition can also be inferred from the difference seen between dog breeds. An artificial selection history for high locomotor capacity selects for one suite of morphological traits and pressure for fighting ability selects for a different suite of traits [27, 28].

The possible tradeoffs between dispersal and foraging and physical competition are one of the more straightforward ones to make predictions about within my system. This is because a great deal of research has established character states and physiological traits that make a species a high quality runner. Males specialized for foraging or dispersing should be morphologically similar to cursorial species, while males specialized for competition should be morphologically similar to other known high-competition species, such as pit bulls or lowland gorillas. For example, dispersal males should have relatively long gracile limbs, while competitively specialized males should be squat and look like “pure awesome.” Physiological specialization should also be present. Cursorial males should be more similar to mice selected for high voluntary running than high competitive ability males. This tradeoff could present itself as difference in muscle fiber type percentages, for instance. Specific predictions can be made from the work of the Garland lab. From an evolutionary perspective, it is possible that efficient running morphs are better if a population is expanding into novel territory, or in low density areas were foraging is important. The opposite also

holds true, in high density populations with males who want to stay close to natal areas, it might be better to be specialized to fight, especially if the population is commensal and humans provide readily available energy sources.

Competitive ability might also tradeoff with the capacity to survive and reproduce within a famine cycle. Larger body size requires absolutely more energy, which can be maintained only during times of normal food distributions or feast years, while smaller body sizes may aid survival and increase fitness during time of famine because they require less absolute energy. This idea is supported by finding is marine iguanas discussed above [29]. In house mice the “boom-bust” cycle of population densities presents the right ecological scenario to select for morphs that are adapted to the low energy part of the cycle [30]. This phenotype would be consistent with the “thrifty” phenotype seen in some humans populations [31]. Specifically, males want to be large to allow them to compete successfully and at the same time want to be small to reduce energy requirement to increase survival probability during famine times. Although, this tradeoff has not been directly tested in house mice, it is known that being dominant in a laboratory setting incurs nontrivial costs, especially if a male is a relatively small dominant. Specifically, relatively small, but still “dominant,” males reach a smaller adult body weight and have slower growth rates [32].

If this tradeoff with social dominance does exist, I can make specific predictions about the physiological characteristics that would be present within each of these morphs. Males specialized for competitive ability will have larger overall body size and a high mass-specific metabolism. The high mass-specific

metabolism is probably going to be driven by a high percentage of lean tissue. Additionally, if there were a tradeoff with locomotor ability a highly competitive male's increased locomotor costs would exacerbate survival difference during time when long distances are required to fulfill bodily energy requirements. It is also possible that males who excel at surviving famine would want to be large. However, these males would not have a high percentage of lean tissue. They would instead have a high percentage of adipose tissue with low metabolic rates.

Immunocompetence is another area where there is a possible tradeoff with dominance ability. It has been experimentally demonstrated that high testosterone promotes high dominance rank in the presence of reproductive resources in house mice [33]. Testosterone is an immunosuppressant [e.g., 34]. The logical extension is that dominant males might have decreased immunocompetence. Following this conclusion, one can easily imagine a situation where a pathogen sweeps through a population and kills any individual that is not able to invest large amounts of resources into an immune response. Dominant males and other compromised individuals would be removed from the population.

Phenotypic trade-offs between competitive ability and immunocompetence might come in several forms. Firstly, one would predict that dominant males would mount less of a response to a standardized infection of an ecologically relevant pathogen. It would be interesting to see if a reduced response is also seen in individuals before they become dominant or only after. In a natural population, one would also expect dominants to die at higher frequency during a

pandemic event. It is also conceivable that tradeoffs exist within specific components of the immune system.

Another interesting tradeoff that might help maintain allele diversity within a house mouse population is antagonistic sexual selection. Briefly, antagonistic sexual selection occurs when alleles that increase one sex's fitness decrease the fitness in the other sex. Within the context my experiment, alleles that increase a male's competitive ability would in turn decrease the fitness of females who contain those alleles. This phenomenon has recently been recognized as a potentially powerful force for maintaining genetic diversity with a given species or population [35, 36].

Although not at the genetic level, female mice that are between two male siblings *in utero* are reproductively compromised compared to sisters born between female siblings. These compromised traits include age at first reproduction, age at menopause, litter size, rate of progeny production, and attractiveness to males [37-39]. However, there is evidence that they have increased competitive ability, which might aid in survival [40]. These studies are suggesting that being male-like is bad for female fecundity, a very natural conclusion. It would be interesting to see if these hormonally driven results are recapitulated with genetic forcings.

Sexual dimorphism is thought to be one solution to antagonistic sexual selection [36]. House mice have a pronounced body size dimorphism. Given the importance of body size to a species biology, this particular dimorphism might be suggesting that male and female house mouse biology are sometime in direct

competition and that this species might have been subjected to extensive sexual conflict in its earlier evolutionary history. Within primates this particular tradeoff, between male and female body mass, has been demonstrated to decrease female birth rates [41].

If antagonistic sexual selection is present within this system, several predictions can be made. Daughters of dominant families should have less progeny, decreased litter mass, lower survival of progeny, and/or decreased time spent performing maternal care (e.g., thermoregulation of the litter, etc.). They might also have larger body size, which should increase time to first reproduction and decrease potential investment in lactation [41]. The reverse of this is that subordinate families would produce high quality daughters and low quality sons. Most of the above predictions simply are reversed in predicting this case.

Interestingly, male mice can also provide a substantial amount of parental care [42]. This is especially true if a male is in a monogamous mating situation [42]. In fact, males do everything that a female does in care of progeny, except nursing pups, and even exceed females in some behaviors, such as nest guarding [42]. It is possible that males that excel at parental duties are of lower competitive ability. Although, fathers do obviously breed and hold territories, this counter selection might not be extreme. If a trade-off does exist with social dominance, it might produce an optimum between the level of paternal care and social dominance ability that would maximize fitness.

Again, if this tradeoff does exist, several trends should be seen. Males that are of high competitive ability should exhibit low parental care. General measures

of parental care should be reduced, such as time to pup retrieval. It would be interesting to see if all measure of parental care are lower or deficient in only some of the paternal care traits are seen.

This introduction provided a general background for the dissertation project described in the following chapters. It was built upon the idea that optimization of a phenotype for social dominance ability in male house mouse is in direct conflict with many other important life history traits. It is important to reiterate that the topics discussed above do not represent an exhaustive list of important life history traits constraining competitive ability.

1.4 Summary of chapters

In Chapter 2, I present the findings from a two-generation experiment that broadly assessed some of the predicted key component of the behavioral physiology that underpins social dominance ability in male house mice. Chapter 3 describes the findings from an experiment meant to assess the relationship between offensive aggression and social dominance ability. Chapter 4 describes our investigation of the relationship between the Major Urinary Proteins (MUPs) and social dominance ability. Chapter 5 details a meta-analysis that tested the prediction that primate species that exhibit the highest levels of male-male competition would also have the largest relative brain size.

The main conclusion of Chapter 2 is that social dominance ability is heritable. This was the first demonstration of additive genetic variation for this trait in this species. Body mass was found to be a moderate influence on social

dominance ability. Male biased litters were found to produce male of lower ability, although this was a very small effect. Litter size, small relative age disparities, order of placement in the competition arenas, and ear punched for identification were not found to influence social dominance ability. In a broader context, that additive genetic variation was found for social dominance ability, the trait primarily responsible for male fitness, suggesting that this trait is under counter selective pressures constraining it from reaching its optimum.

The main conclusion of Chapter 3 is that offensive aggression and social dominance ability are two different phenomena. The intensity of aggression displayed in a resident-intruder test was not predictive of ability in the competition arena against other wild mice. However, the most competitive males were more likely to attack an intruder. This effect was not significant but was suggestive. Body mass disparity between the resident and intruder was not predictive of whether an individual attack or the intensity of aggression displayed in the test. None of the measures of aggression from the resident-intruder test demonstrated significant heritabilities. From the data, we suggest that aggression might be a signal of low competitive males that they are willing to “go all in,” which might allow them to win encounters. The data also suggest caution when interpreting contrived lab tests that remove subjects from the evolutionary context of the behaviors being tested.

The main conclusion of Chapter 4 is that MUPs do not signal social dominance ability via their concentration in urine. MUP concentration was found to respond to time spent in a social environment by increasing. Body mass

negatively influenced MUP concentration. Creatinine was assessed for its suitability as a control measure to standardize MUP concentration between subjects. Additionally, MUPs were positively influenced by body mass and negatively influenced by increased experience in a social situation. Our data suggest that both of these factors need to be accounted for when analyzing creatinine controlled MUP concentration. A future direction of this research would be to assess the relationship between social dominance ability and the ligands associated with MUPs.

The main conclusion of Chapter 5 is that primate species that exhibit the high levels of male-male competition also have females with the largest relative brain sizes. The results of male brain size were not strongly in favor of this hypothesis, however, did not produce many results to direct contradict the hypothesis. A future direction of this study is to assess more variables known to influence primate brain size, which will help apportion variation into more causal components.

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CHAPTER 2

COMPETITIVE ABILITY IN MALE HOUSE MICE (*MUS MUSCULUS*): A MULTIFACTORIAL TRAIT

2.1 Abstract

Males of many animal species physically compete for resources. Despite its evolutionary importance, specific traits that increase competitive ability have rarely been addressed. Here we investigate multiple factors that possibly underpin the ability of quasi-wild male house mice to be highly competitive. Using a two-round tournament within seminatural enclosures, we measured a male's ability to gain and hold a resource. Body size had a moderate, positive influence on competitive ability. While controlling for the influence of body mass, competitive ability was highly heritable suggesting that other traits are also contributing to this trait. Interestingly, litter sex ratio had a weak negative influence on competitive ability with individuals from male-biased litters being less competitive. Collectively, our results suggest that competitive ability is a complex phenotype. Our study also suggests how little is known about the proximal causes of competitive ability, one of the most important known behaviors in the animal kingdom.

2.2 Introduction

Intense, physical competition among conspecific animals is ubiquitous, despite severe costs [1, 12, 43-45]. The importance of physical conflict to anatomy, behavior, and life history traits has been recognized since Darwin [46]. Large investments into traits that increase competitive ability attest to its importance [43]. Generally, the outcome of physical conflict based on differences in competitive ability leads to differential access to reproductive resources [1, 11, 12, 44]. Indeed, competitive ability's most germane evolutionary feature is individuals with high competitive ability are the fittest on average [1, 10, 44, 47-50]. The phylogenetic diversity of the species with intense male-male competition reflects its importance [43], and highlights the need to understand more about the physiological, behavioral, and evolutionary basis of competitive ability.

Despite its overwhelming importance, the factors influencing competitive ability remain largely unexplored. One exception to this statement is body mass. Relatively large body mass has long been thought to be a result of selection for increased competitive ability [5, 46, 51, 52] and body size has been demonstrated to strongly, positively influence competitive ability in many species, including house mice [e.g., 5, 6, 10, 26, 53, 54]. To address this need, we preformed an experiment designed to emphasize male-male competition while trying to approximate the physical environment of commensal house mice. This was achieved by manipulating the sex ratio and density of males compared to observed commensal populations using seminatural enclosures. We investigate the possible influence of several measures of body size, genetic (additive genetic

variance, genetic correlations), and demographic (litter size, litter sex ratio) components on competitive ability.

House mice (*Mus musculus*) are ideal suited for the study of male-male competition for several reasons. Most importantly, competitive ability plays a critical role in the reproductive success of male house mice [1, 10, 44, 55]. Male-male competition is responsible for creating the polygynous social system in which males physical compete for resources forming demes [13, 14, 56], which usually includes several breeding females and some offspring [13, 15]. Accordingly, when males are placed in laboratory seminatural enclosures fighting is intense [16, 17], with a clear social network established via rapid deme formation [15-20, 57]. Resource holding males are fittest in seminatural enclosures [20, 55]. Thus, we define “competitive ability” within male house mice as the capacity to gain and hold resources by repeatedly winning physical conflicts. This term is synonymous with resource holding potential [58].

We examined the influence of two demographic factors on competitive ability: litter size and litter sex ratio. Litter size is negatively correlated with weaning weight in multiple species of rodents, including house mice [54, 59-61]. Weaning weight is positively correlated with adult body weight, which is positively correlated with competitive ability within house mice [54]. For these reasons, we predict that increasing litter size negatively influences competitive ability.

Sex composition of litters has the potential to influence competitive ability through several possible mechanisms. Litter sex ratio can influence competitive ability either through pre- or postnatal effects. One of the most likely mechanisms

for a prenatal influence is mediated through *in utero* position. Within female biased litters, males have a higher probability of being between two females. Differences of *in utero* position lead to different localized hormonal environments, which cause many well-established phenotypic effects [37-40, 62]. Here, potentially important effects are decreased levels of aggression in males between two sisters [62]. However, no difference of *in utero* testosterone concentration was seen for males between two sisters *versus* two brothers [37]. Interestingly, females displayed increased competitive ability when they developed between two brothers [40, 62], although it was not a strong effect. Any effect observed due to litter sex ratio can also be mediated through postnatal effects. Competition for care against primarily brothers may lead to a different phenotype than competition against mainly sisters. To try to tease these two possibilities apart, we used several different measures of litter sex composition: sex ratio, number of male siblings, number of female siblings, and if there is an interaction between litter size and litter sex ratio.

The protocol used here also provides an opportunity to ask if an individual's competitive ability is influenced by different factors when faced with different levels of competition. For example, we may ask how does body mass influence competitive ability globally, within the first round when competitors were randomized, in winner groupings with an increased level of competition, and in loser groupings with a decreased level of competition? Each of our questions was analyzed this way to try to elucidate all patterns.

Our study was conducted to investigate possible traits that underpin competitive ability in male house mice. To this end, two generations of mice were grouped into multimale/single female groups and placed into seminatural arenas. Possession of a resource (a preferred nesting site) during a 3 day trial was used to assess competitive ability. Firstly, we investigated the influence of body mass using several different analyses. Next, we estimated the additive genetic variation of competitive ability of male house mice. Heritability was estimated using a 13-generation pedigree-based analysis. Finally, the effects of two litter demographics-- litter size, litter sex ratio-- were also investigated.

2.3 Methods

2.2.1 Subjects

House mice were obtained from a colony maintained at the Department of Biology, University of Utah. The population founders were wild mice and a circular breeding program has maintained the colony through 11 generations, which includes a comprehensive pedigree. The inbreeding coefficient (average pairwise consanguinity) of this population was 0.0839, which is comparable to the only to estimate of this parameter from a wild population [63]. Importantly, mice from this population have already been used in many behavioral and genetic studies on the major histocompatibility complex [56], inbreeding depression [64, 65], mating preferences [66, 67], kin recognition [68], sexual selection [69, 70], the interplay of disease resistance, hormones and sexual

attractiveness [66, 71], and fitness consequences of infectious and genetic disease [55, 71, 72] .

This experiment was originally attempted with an outbred laboratory strain, Hsd:ICR. Our observations from two trials indicate that these mice lack the behavioral skills to develop a typical hierarchy. Even though a female was present, males exhibited little physical competition and co-habitated together with the female in the preferred nesting site. However, by the end of both trials, all individuals (including the female) except one male were removed due to excessive stress. These behaviors were not seen with our quasi-wild colony trials. Based on these observations, we suspect that many or most outbred laboratory strains may be too far removed from wild-type to be biologically relevant for realistic behavioral experiments.

2.2.2 Breeding Protocol and Animal Husbandry

Thirty-six breeding pairs were used to found the first generation (92 males, 93 females) of the current study, termed g1. All male mice that completed the two round tournament were randomly mated to sisters of other competing males to generate the second generation, termed “g2.” Pairings excluded both siblings and cousins mating to avoid inbreeding, which has been shown to have major impacts on house mouse fitness [64, 65].

Male of this colony regularly exhibit postweaning aggression when housed together; therefore, it is likely that filial dominance networks would have been established. To remove the possible confounding effects from intralitter

dominance networks, which likely would have precipitated winner/loser effects, individuals were singly housed. Individual housing also controls for differences in the number of littermates that would be present if males were housed in family groups. Males were housed individually starting at weaning; females were housed with sisters in standard cages. Although this solitary housing is unlikely in nature, it is the lesser of two evils. In addition, males are likely solitary for some time after dispersal until they are accepted as subordinate in an established territory or usurp a previously resource holding male from his territory. Animals were housed according to standard protocols under a 12:12h light:dark cycle with standard rodent chow and water available *ad libitum*. All protocols were approved by the IACUC, University of Utah.

Although we are unaware of any studies directly addressing this point, we find it unlikely that males would be sexually experienced as they disperse from their natal litters and attempt to depose an established territorial male. We believe this for two main reasons. Mice are known to avoid inbreeding [65] and the dominant male of a deme increases aggressive acts towards male offspring as they mature [73]. Because of this, we choose to keep our males as virgins entering into their first competition round. We purposely did not control for possible differences in sexual experience during the second round. This was for several reasons. First, males that are fighting for territories are likely to have had differing sexual histories. Second, mating is known to cause several serious hormonal shifts [74, 75], which might have confounded the effects from our emphasis on male-male competition.

2.2.3 Competition Arenas

Arena design was based on the established “phenotron model system” developed by the Potts laboratory. This system was first used to discover MHC-based mating and nesting preferences in wild mice [56, 76]. Acrylic sheeting was used to construct small seminatural arenas in which the male-male competition was staged; dimensions 140 x 30 x 15cm [Fig. 1]. The arena’s design is based on the preference of house mice to maintain territories that include secluded, dark nest sites that offer protection from predators and infanticidal conspecifics [19, 20, 77, 78]. Thus, each of the arenas consisted of two chambers: 1) a preferred nesting territory and 2) a much larger communal area that represents a suboptimal territory. The preferred nesting site had opaque walls and ceiling, food and water, and nesting material (paper towels); dimensions 15 x 30 x 15cm. The larger communal area had transparent walls and ceiling, shared food and water, and no nesting material. A remotely operated, sliding electric door allowed researchers to control access to the preferred site. Access was blocked before a researcher entered the testing room to facilitate identification of the male occupying the preferred site.

2.2.4 Preparation Procedures

The randomly selected female mice that were used to stimulate competition in the dominance trials were separated into individual cages 2 weeks before the beginning of each round of competition [48]. Females used in the arenas were not bred afterwards. Passive integrated transponders were inserted

into all g2 males to help more rapidly identify them during the competition rounds using a hand-held receiver.

2.2.5 Testing Procedures

Dominance trials were completed for both the first (g1) and second (g2) generations, and consisted of 2 “competition rounds.” At the start of each competition round, four males and one female were randomly assigned to an arena. (Note: No siblings were grouped together.) This is a density of 10 males/m^2. This is roughly 1.5-2.5x the density that has been reported for commensal populations and wild-derived colonies in large enclosures with self-regulated growth [79, 80]. This is also about 4x a male biased sex ratio as reported from commensal populations [81]. All mice were inspected to ensure that they had not developed conspicuous abnormalities or injuries since weaning. All mice were then weighed, ear punched (for identification), and placed within the arenas. The order males were placed in the arenas and which ear(s) were punched was random. Mice were weighed at the end of the competition round. Between the two competition rounds, males were given 6 weeks recovery. During the second competition round, males that won the first round were placed with other winning males and first round losers with other losers. Because some males were removed prematurely from the first competition round, only three males and one female were placed in each arena for the second competition round. Mice that had competed against each other in the first round or had

identical ear punches were excluded from being opponents during the second round of competition.

Mice of g1 were eight months and g2 mice were 5 months old entering the dominance trials. Full bodily maturity is hard to predict for any population of mice; however, it is known that males from other wild-derived colonies still have positive skeletal growth patterns and do not fully sexually mature until at least 90 days [82]. Females of wild, commensal-derived colonies have been documented to reach sexual maturity at 59 ± 24 days, meaning one has to likely wait > 3 months to ensure that the vast majority of females are sexual mature [83]. Also, males disperse from natal litters when they are ~3 months of age [73]. For these reasons, our mice were not tested before 4 months of age. Several logistical challenges caused the delay in testing g1. Importantly, these ages are easily considered young adult for wild-derived colonies kept in laboratory conditions [83, 84]. Additionally, if there were an interaction between genotypes and age such that different genotypes “peaked” in competitive ability at different ages, then we would lose any signal of causal factors.

To begin the second dominance trial of the second generation, males were sorted according to their father’s competitive ability. One male from a two time winning sire, two males from different single winning sires, and one male from a zero winning sire were randomly assigned to an arena with one female. Competition rounds for g2 mice were completed using the same protocol as the g1 mice.

We did not randomize males for the second round of competition for several reasons. First, it would have ignored the winner/loser effects that precipitate large changes in hormonal [e.g., 75, 85, 86, 87], gene expression [88], and behavioral measures in mice and other rodents [e.g., 75, 89]; and those are just the known effects. Second, our experiment was designed to emphasize competitive ability. It would not have been useful to know that first-round winners beat first-round losers in the second round; we already had evidence they were of higher competitive ability. Third, although the first and second rounds are not independent, randomizing the males would not have solved this problem. In fact, because we realized that the rounds were not independent, we place winners with winners, etc. Additionally, competing males in a third round is unlikely to have changed the results qualitatively. This is mainly due to the use of the animal model, which weights the entire pedigree worth of information in predicting heritability. There was a nonrandom sample of 2-time winners and 2-time losers. Had we gone a third round, there would have likely been families that produced more 3-time winners than expected on chance and the same with 3-time losers.

Competition rounds lasted 3 days. The three days time limit was chosen based on preliminary experiments, which suggested that an unacceptable amount of males had to be removed due to duress (body mass loss, extensive wounding, etc.) if the trials were of longer length, such as 7 days. Between 6 and 12 observations were made to determine which male occupied the preferred nesting the most over this period. The number of observation was tailored to each trial to minimize disturbance, while still providing consistent evidence of

competitive ability. At least one observation per day was during the night resting period. Possession of the preferred nesting territory was considered evidence that a male was dominant and thus highly competitive. Additionally, we used an established measure of competitive ability, wounding [10]. This measure was quantified for all rounds, except the first round of generation one. Wounding was scored separately for the tail and hind body region. This is not the first study to use possession of preferred territory and wounding as measures of competitive ability in the absence of direct behavioral observations [e.g., 10, 48]. Mice that received a conspicuous injury or appeared under serious stress were removed from the arenas immediately. Males that were removed due to apparent stress were excluded from the second competition round.

2.2.6 Starting Populations of each Generation

Generation 1 contained 80 males placed into 20 competition arenas. Generation 2 contained 48 males placed into 12 competition arenas. One hundred seventeen males were completely phenotyped. Several males were dropped completely from the analysis for various methodical reasons. For example, the second competition round was between three males and there was not always a multiple of three males left from the first round. Therefore, several males that participated in round one were not competed in round two and were not completely phenotyped.

2.2.7 Scoring Regime

Competitive ability was scored by assigning each male the number of rounds he won. For example, males that won the first round but lost the second or males that lost the first round but won the second round were each assigned a score of 1. This trait was considered ordinal. As such they can be analyzed with a linear mixed model if the distribution is not too skewed [90]. Skewness of our scores was checked before analysis.

2.2.8 Statistical Analyses

Multiple methods were used to address each hypothesis or question asked. This was done in an attempt to produce robust results that are not dependent on a particular method. Where parametric methods are used, we also provide nonparametric results where possible to support our conclusion.

Heritability was estimated using the “Animal Model.” This method utilizes pedigree-based relationship information and linear mixed model to partition observed variance into causal components (i.e., genetic, environmental) [91, 92]. Using all available relationships greatly increases power to estimate additive genetic variance [92, 93]. It should be noted that sample size within this study is relatively small and thus constrains our ability to utilize more comprehensive quantitative genetic methods [92]. Consequently, we have focused on testing some specific hypotheses and discussing the implications [92]. We used several different methods to assess the amount of additive genetic variation in competitive ability, both raw and body mass controlled. We also assessed the

genetic relationship between body mass and competitive ability by estimating the genetic correlation between the two. The raw heritability of competitive ability was assessed with the “regress” package [94] of R with significance established by a permutation test Heritability of body mass was also calculated using this method [95]. A body mass controlled measure was estimated with from the R package “MCMCglmm” [96]. With body mass as a fixed effect, we used a Poisson distribution to model competitive ability and ran the model through one million iterations. This model also produced an alternative measure of body mass’s influence on competitive ability and provided an opportunity to assess if other traits are involved with competitive ability. Body mass was the only factor found to influence significantly competitive ability; therefore, we report only those results. However, all other factors were tested in this model for significance. The genetic correlation (r_G) between raw scores of competitive ability and body mass was also estimated with a Markov Chain Monte Carlo algorithm; using a poisson and normal distribution, respectively, in a bivariate animal model; of the MCMCglmm package [96] to estimate the amount of common genetic controls.

The correlation between body mass and competitive ability was estimated using a Pearson’s correlation (significance established with permutation test), and a Spearman’s rank correlation. Furthermore, to better elucidate the influence from size, a male’s body mass was divided by the mean of his fellow competitors. This “body mass disparity measure” was then plotted against success with each competition round and evaluated with a logistic regression.

Competitive ability was regressed against each male's natal litter size. In addition, to validate all our assumptions, adult body mass was correlated (Pearson and Spearman's rank) with weaning weight and litter size with weaning weight. Weaning weight was only available from g2.

Litter sex ratio was scored as a percentage (# of males / # of progeny). This measure was then regressed against competitive ability.

The interaction between litter size and litter sex ratio was assessed with a linear regression.

Three possible confounding factors were analyzed; the ear that an individual had punched for identification, the order that a male was placed into his first competition round, and relative age of the competitors. Fisher's Exact test was used to analyze the ear and placement data. Relative age was scored as days younger than the oldest males and correlated with competitive ability. No confounding influences were detected (ear punched: p= 0.19; placement: p= 0.55; relative age: p= 0.81).

All analyses were conducted in R [97].

2.4 Results

2.4.1 General Descriptive Results

The mean body mass change between the start and completion of a round was -5.2%. The mean body mass change for losing individuals was – 6.8% and a winning individual was -2.2%. An average of 20% of males had to be removed

from each competition round due to excessive stress (body mass loss, extensive superficial wounding, etc.).

2.4.2 Body Mass: Descriptive Results, Influence on Competitive Ability and Heritability

The mean body mass of males in our population at the beginning of tournament was 21.4g (SD: 2.7g) and mean weaning weight was 13.0 (SD: 1.7). Also, the generations did not differ in body weight entering the dominance trials (t-test: $p > 0.1$; Wilcox test: $p > 0.1$).

Body mass was heritable, $h^2 = 0.82$ ($p < 0.001$; n= 117; **Table 2.1**) and was significantly correlated with competitive ability globally, $r = 0.33$ ($p < 0.001$; $\rho = 0.34$, $p < 0.001$; **Figure 2.1a**). Interestingly, 2-time winners were between 22.7g and 27.1g (**Figure 2.1a**). Additionally, in only 29 of 62 (47%) competition arenas did the largest male win. The body mass disparity measure between opponents within a single competition arena (body mass of an individual male/mean body mass of his opponents), was significantly related to success, slope= 10.81 ($p < 0.001$, n= 128; **Figure 2.1b**). Body mass had a significant positive influence on competitive ability in the multivariate heritability model, (slope= 0.163, $p = 0.0032$; **Table 2.1**). Finally, there was a genetic correlation (r_G) between competitive ability and body mass of 0.66 (95% CI's: 0.016, 0.88; $p = 0.05$; **Table 2.1**).

2.4.3 Correlation between Two Measures of Competitive Ability

Possession of the preferred territory and the least wounded male within each enclosure was identical in all but two of the trials (96.7%). Our measure of competitive ability was significantly negatively correlated with wounding (g1: ρ= -0.53, p <0.001; g2: ρ= -0.47, p= 0.003; both: ρ= -0.50, p <0.001).

2.4.4 Heritability of Competitive Ability

Raw competitive ability was found to have a high narrow sense heritability, h^2=0.62 (p < 0.005; n=117). Body mass controlled competitive ability was also heritable, h^2= 0.56 (95% CI's: 0.216-0.938; p <0.01; n= 117; **Table 2.1**).

2.4.5 Demographic Factors: Influences of Litter Size and Litter Sex Ratio

Weaning weight was significantly correlated with adult body size, as expected (r= 0.66, p < 0.001; ρ= 0.65, p < 0.001; n= 46). However, weaning weight was not correlated with litter size (r= 0.19, p= 0.11; ρ= 0.17, p= 0.27). Competitive ability was not influenced by litter size globally (slope= -0.015, p= 0.74; ρ= 0.1, p= 0.27). Litter size was also not associated with competitive ability if the data is broken down by round and win/loss groupings (Round 1: slope= 0.11, p= 0.37; Round 2: winners- slope= -0.26, p= 0.20; losers- slope= 0.15, p= 0.35).

Litter sex ratio was negatively correlated with competitive ability, r= -0.22 (p= 0.02, n= 117; ρ= -0.18, p= 0.053; **Figure 2.2**), but was not a significant factor when included in an animal model. Competitive ability from round 1 alone is significantly negatively associated with litter sex ratio (slope= -2.78, p= 0.02). Litter sex ratio was not associated with winning in the second round (winners: slope= -2.09, p= 0.42; losers: slope= -0.73, p= 0.55). Number of male siblings was not correlated with competitive ability (ρ= -0.07, p= 0.45), but number of sisters was (ρ= 0.19, p= 0.042).

There was no interaction between natal litter size and sex ratio (r^2= 0.05, p= 0.14).

2.5 Discussion

We performed an experiment to investigate the proximal cause of competitive ability in male house mice. We manipulate both the density and sex ratio of commensal house mice to increase the level of male-male competition using a wild-derived colony in seminatural enclosures. We simultaneously investigated several possible factors that influence competitive ability in male house mice. This is the first study to estimate the influence of multiple factors underpinning competitive ability from such disparate areas of life history at the same time. Our main conclusion is that body mass had a surprisingly moderate influence on competitive ability (**Figure 2.1**, **Table 2.1**). Our analysis of body mass using several different measures never explained much variation. This conclusion is also supported by the heritability analysis that demonstrated that

competitive ability has a large additive genetic component, even while controlling for the influences of body mass (**Table 2.1**). Litter sex ratio also modestly influenced competition ability (**Figure 2.2**). Interestingly, there was stronger influence from the number of sisters and no influence from the number of brothers. Under the conditions of this study, litter size did not affect competitive ability and there was no interaction between litter size and litter sex ratio. The results collectively support our suggestion that competitive ability is a complex phenotype with many traits interacting to determine its value.

Body size was moderately, positively correlated with competitive ability globally and explained 11% of the variation in competitive ability (**Figure 2.1**, **Table 2.1**). Importantly, the mean and standard deviation of body mass of our population is directly comparable to several reported measures from wild and wild-derived populations suggesting that we are capturing evolutionarily relevant range of body size [98]. Several interesting trends emerged. First, the largest males in this study were of low competitive ability. Second, 2-time winning males were 48 to 81% larger than the smallest male; indicating that top competitors are within a fairly narrow range of body mass (**Figure 2.1a**). This range makes some physiological and biomechanical sense. It is possible that the largest individuals were simply obese, which would handicap them because as an individual's body mass increases, the weight to strength ratio decreases. However, if a male is too small then the greater weight and strength of his opponent will be a significant advantage. The body mass disparity measure also indicates that body mass significantly influences competitive ability. Males who won a particular

competition round where on average larger than the mean body mass of their opponents; however, many individuals who were larger than their average opponent lost (**Figure 2.1b**). Again, this suggests that while relative large body mass is advantageous, it is by no means the only trait involved in competitive ability. Our heritability analysis gives a very similar conclusion to the direct measures of the influence of body mass. We found a large amount of additive genetic variation after controlling for the influence of body mass suggesting that competitive ability is determined by more traits than body mass. We found a genetic correlation of 0.66 between competitive ability and body mass. This again suggests that the two traits share some common genetic basis, but are not synonymous (**Table 2.1**). Our test results are also supported by a qualitative comparison of the body size of the winners and losers within individual arenas. The largest male within an arena only won 29 out of 62 trials (47%), suggesting that other characters have a substantial influence on an individual's ability to win physical conflicts. Collectively, our results suggest two things. First, there is substantial variation that body size does not account for in competitive ability. Second, there is a body size optimum for physical conflict.

Given the reproductive advantages of occupying the top of a social hierarchy, it is not surprising that competitive ability is often similar in parents and offspring. Here, we found a high heritability for competitive ability (**Table 2.1**). The heritability of competitive ability has also been estimated in five species: paradise fish, speckled cockroach, Japanese quail, chickens, and deer mice [49, 99-102]. Consistent with our results, competitive ability was found to be heritable

in all of the studies. Some authors have suggested that competitive ability cannot evolve because it is a trait derived from the interaction of multiple individuals [103-105]. However, competitive ability can prove heritable because of indirect genetic effects, even if a social environment is necessary for expression [100, 106-109]. That competitive ability demonstrated heritability suggests that is likely be under counter-selecting pressures from other life-history traits, such as locomotor efficiency, which might help maintain genetic variation [28].

Litter sex ratio was marginally, negatively correlated with competitive ability depending on the statistical test (**Figure 2.2)**. Effects from litter sex ratio can be caused by pre- or postnatal influences. The most likely pre-natal influence would be from *in utero* hormonal environment. In our study as the proportion of male progeny increased, males demonstrated lower competitive ability. Although not a large effect, high testosterone *in utero* increased female house mice competitive ability [40], which supports the possibility that *in utero* environments affect competitive ability. It could also be that litter sex ratio effects are mediated through postnatal interactions with siblings. It is possible that as a litter becomes male biased there is more competition for direct care. We assessed whether the number of male or female siblings better correlated with competitive ability. Only number of female siblings significantly correlated with competitive ability. This suggests that our effect is mediated more through sisters than brothers and supports a postnatal interpretation of our results. There was no interaction between litter size and litter sex ratio. Finally, litter sex ratio was not retained as

predictive factor in the multivariate animal model. We would like to emphasize that this is a very weak effect, which is only marginally significant.

Litter size did not have a detectable effect on competitive ability. This result is surprising given the high metabolic demand large litters place on the nursing mother [61]. Although, given the moderate effect that body size has on competitive ability, finding no link between the two variables seems plausible. It is possible that dames were able to compensate for large litters because of the high quality *ad libitum* food and water. The nonsignificant positive trend between litter size and weaning weight supports this possibility.

This study highlights the need to attempt to create realistic environments when studying evolutionarily important behaviors, even when manipulating one component of a system like we did here with male-male competition. Many studies about competitive ability are not designed around the evolutionary context of why individuals fight, resources. There is no reason to fight and risk injury if there will be no reward for such behavior. Illustrating this point, Zielinski and Vandenbergh [33] manipulated the testosterone levels (T) of male mice to generate either high or low testosterone males. When tested over 2 days in a laboratory, the two classes of males showed no significant differences in their competitive ability. However, when tested against each other in the presence of a female, high T males won 11 out of 14 (78.5%) trials. Gray et al. [110] also found males are more likely to engage in agonistic encounters in the presence of resources. Our study along with previous work demonstrates the need to

consider carefully house mouse evolution when designing experiments about life history traits.

In conclusion, our study demonstrates that a surprising mix of factors influences competitive ability within male house mice. Body mass was found to have a surprisingly modest influence on competitive ability. This suggests that, while body mass is associated with competitive ability, other traits are also important. Competitive ability also exhibited large heritable variation, even after controlling for influences of body mass. Litter size and relative age disparity were found to have no effect on competitive ability, despite strong reasons for predicting possible influence from litter size. Litter sex ratio was found to slightly influence competitive ability with males who have a high proportion of brothers not competing as well, contrary to expectations. These findings suggest a complex and interesting foundation of competitive ability. Heritable variation remaining in this population also suggests that traits underlying competitive ability may be subject to counter-acting selection, resulting in tradeoffs, thus maintaining variability.

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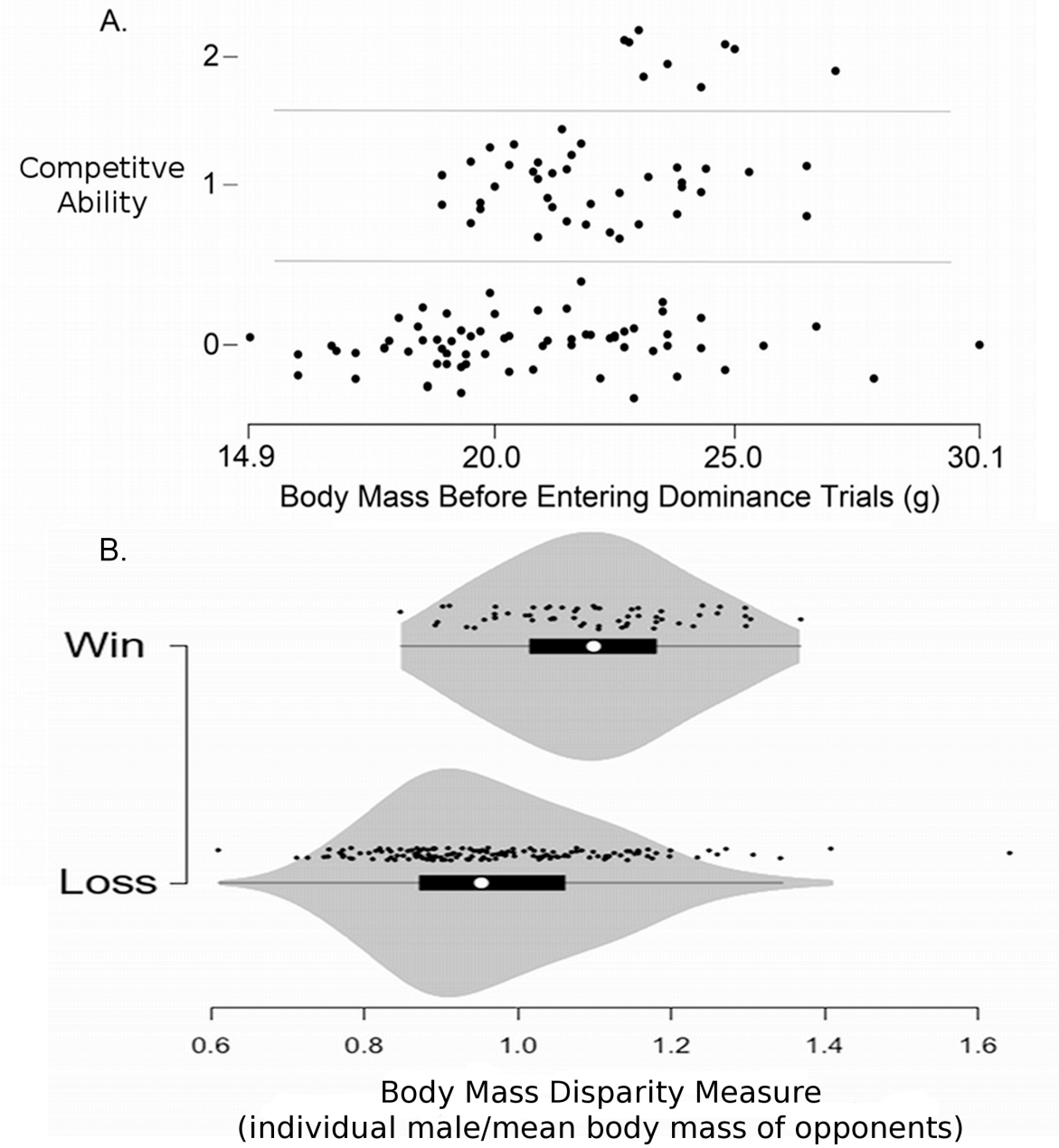

Figure 2.1. Graphical analysis of the influence of body mass on competitive ability. (A) Male body mass entering into his first competition round (g) *versus* competitive ability (# of competition rounds won) (r= 0.33, p= <0.0003). Random noise was added to the y-values to help visualize the data. (B) Body mass disparity (body mass of the subject male/mean opponent body mass) within a competition round *versus* success within that round. Random noise was added to the y-values to help visualize the data. A logistic regression tested for a significant trend (slope= 5.78, p= <0.001, n=204).

Table 2.1. Quantitative Genetics of Competitive Ability.

Trait	Magnitude	95% CI	n	*P*
Animal Model: Body Mass α V_A				
V_A/V_P	0.827	(.45-0.947)	117	<0.01
Animal Model: CA α V_A				
V_A/V_P	0.58	(0.23-0.92)	117	<0.01
Animal Model: CA α V_A (1st Round Data Only)				
V_A/V_P	0.51	(0.16-0.80)	117	<0.01
Genetic Correlation: CA α Body Mass				
Body Mass	0.66	(0.016-0.889)	117	0.05

Estimates were generated using the 'animal model,' see methods for details. CI= Confidence Intervals, n= Sample Size, V_A= Additive Genetic Variance, V_P= Total Phenotypic Variance, CA= Competitive ability.

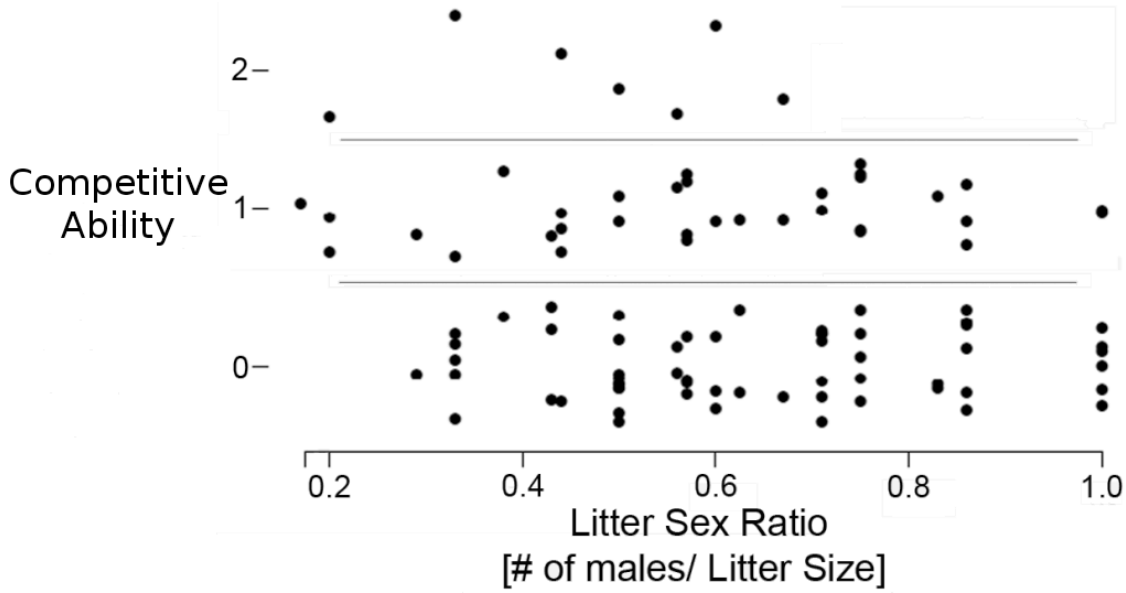

Figure 2.2. Graphical representation of the influence of litter sex ratio on competitive ability. Litter sex ratio (# of males/# of progeny) *versus* competitive ability (# of competition rounds won) (r= -0.22, p= 0.02; ρ= -0.18, p= 0.053). Random noise was added to the y-values to help visualize the data.

CHAPTER 3

AGGRESSION AND COMPETITIVE ABILITY ARE DIFFERENT PHENOMENA IN MALE HOUSE MICE

3.1 Abstract

Ability to perform well in physical competition is important to fitness in many animals. However, little is known about the traits that underpin competitive ability. Aggression has long been assumed an important component of winning physical competition. Across our tested population, little correlation was found between aggression measured with a standard resident-intruder test and competitive ability assessed with a multiday trial in a seminatural enclosure. Among the individuals who did attack during the resident-intruder test, intensity of aggression was negatively correlated with competitive ability. Additionally, the difference in body mass between the resident and intruder did not influence the decision to attack nor the intensity of aggression seen. However, males with high competitive ability were more likely to attack, although less intensely. Our results demonstrate that in house mice, which have a representative mammalian mating system, aggression is not predictive of competitive ability. This study also cautions against testing complex behavior (e.g., competitive ability) with short,

contrived assays that ignore the evolutionary context of behavior and organism being assessed. In conclusion, our study demonstrates that aggression and competitive ability are not synonymous and further elucidates the behavioral physiology of competitive ability.

3.2 Introduction

The ability to win physical conflicts is essential to fitness in many animal species, especially for males [1-3]. Although conflicts can be costly and dangerous, individuals of high competitive ability overwhelmingly have the greatest reproductive success [2-6]. Competitive ability is an extremely important trait to male house mice due to the polygynous social system in which males engage in physical competition to gain prime territories and thus fitness [1, 7, 8]. Paternity in relation to competitive ability (i.e., cumulative outcome of agonistic encounters) has never been investigated in the wild. However, in seminatural arenas, female house mice mate primarily or exclusively with highly competitive males [1, 9-11]. Consequently, competitive ability represents one of the most important traits that determine a male house mouse's fitness.

Offensive aggression is defined as aggressive acts in competition over reproductive resources, not in defense of bodily integrity [12]. Here, offensive aggression was investigated in the absence of previous social experiences over resources (i.e., offensive aggression of naïve individuals) [12]. Competitive ability is the capability to acquire and hold resources through repeated physical

contests against rivals. We were interested in how offensive aggression relates to competitive ability.

Several studies have looked at the relationship between aggression and competitive ability within house mice. Benton et al. [13] found that the dominant in a group of three males in standard colony cages spent more time investigating/fighting an intruder than subordinates in a separate resident/intruder test in a laboratory strain. Rolland and coworkers [14] found that aggression from a resident-intruder test positively correlated with competitive ability using a neutral arena test. Horn [15] placed four inbreed strains into seminatural arenas. They found that the most aggressive of the four strains sired the most progeny. Oakeshott [16] placed inbred laboratory mice into seminatural arenas and found that the results from a separate neutral arena aggression test was correlated with an individual's competitive ability in the seminatural arena. However, aggression was measured after the competitive ability of an individual was established and so this result is best interpreted as the influence of competitive ability on aggression [16]. While the Benton et al. [13], Rolland et al. [14], and Horn [15] studies suggest that aggression and competitive ability are indeed the same phenomena or highly positively correlated within mice aggression tests using nonaggressive, genetically standardized opponents and competitive ability assessed through outcomes of trials in seminatural arenas have yet to be correlated.

Our study was also able to investigate factors that might influence an individual's decision to attack or that control the intensity of aggression against

an intruder. Offensive aggression could possibly be influenced by many factors other than an individual's competitive ability (e.g., resident's body mass, intruder's body mass, the disparity between the two body masses, the resident's natal litter size or litter sex ratio). Both the absolute body mass and difference among competitors have been demonstrated to influence competitive ability and therefore could influence an individual's decision to attack an opponent [17]. Litter size has been suggested to influence competitive ability, although not in this population [16-18]. Litter sex ratio has also been suggested to influence competitive ability [17]. Therefore, it is possible that these two litter demographics could influence aggressive behavior toward an intruder. All of these factors were assessed to elucidate their influences on aggression.

Competitive ability is the most important, known trait affecting a male house mouse's fitness. Many studies have assumed that the tendency to begin an agonistic encounter is directly representative of the ability to win the encounter. This assumption has underpinned most of the research into competitive ability within the house mouse model system. We directly assessed the relationship between aggression and competitive ability. Mice were first tested using a standard assay of aggression, the resident-intruder test. They were the tested with an ecologically relevant test for competitive ability, the capability to hold a preferred territory over a multiday trial in seminatural enclosures containing resources, including females. This is the first direct test of this assumption in a mammal using separate measures for aggression and

competitive ability, where competitive ability is not based on aggressive acts. Factors that possibly influence aggressive behaviors were also investigated.

3.3 Methods

3.3.1 Subjects

Out-bred, recently wild-derived house mice (*Mus musculus*) were obtained from a colony maintained by Dr. Wayne Potts, University of Utah. The colony has been outbred to purposely maintain genetic diversity and our population has been calculated to have the same average pairwise consanguinity as a wild, interacting population [17, 19]. Standard housing and care protocols were used; see Cunningham et al. [17] for a full description. The University of Utah IACUC approved all protocols.

3.3.2 Aggression Apparatus

We followed a general resident-intruder protocol using non-aggressive C57/J6 males (a standard intruder strain) as opponents approximately two weeks before the first competition round [20, 21]. Briefly, focal males were left in their home cages undisturbed for 1 week. Trials were video recorded and seven minutes long. A barrier was placed between the resident and intruder to start; the encounter began once the barrier was raised. Mice were removed if the resident's attacks became too vigorous. The weight of the mice was taken immediately after the trial.

3.3.3 Video Analysis

One observer, who was blind to all information about focal males, scored all videos. Two measures were scored; latency to first attack and total number of observed attacks. We used the standard definition of each behavior [20]. Briefly, latency to first attack is the time, in seconds, from the beginning of the trial to the first attack. Attacks were attempts to or successful bites of an opponent. These behaviors were chosen not because they are the only important behaviors, but rather they are the most conspicuous, easily scored, and deliberate acts of aggression.

3.3.4 Competition Arenas

Competitions were staged in seminatural enclosures made of acrylic; 140 x 60 x 15cm; based on the established “phenotron model system”, which utilizes the preference of mice to maintain territories that include dark, isolated nest sites to promote competition [9, 11, 22, 23]. Briefly, arenas were divided into: (1) a preferred nesting territory and (2) a larger suboptimal area. The preferred site was dark, contained food and water, and nesting material; 15 x 30 x 15cm. The larger area was transparent, communal food and water, and no nesting material. A remotely operated door allowed physical separation of the two areas, which was done before entering the testing room.

3.3.5 Preparation Procedures

Female mice, used to promote competition, were randomly selected and separated into single cages 2 weeks before beginning each competition round. Passive integrated transponders were inserted into the males 1 week before the trials for identification. Groupings for the first competition round were randomly generated (excluding siblings, male or female) from lists based on a male's sire's competitive ability. Each group consisted of 4 males. The groups included one son from a 2-time winning, two sons from 1-time winning, and one son from a 0-time winning sire. The father's competitive ability was established by the same protocol, details in Cunningham et al. [17].

3.3.6 Testing Procedures

The tournament consisted of two "competition rounds." Four males and one female were randomly grouped. All males were ear punched (for identification) and placed in the arenas. The order of placement and which ear(s) punched were randomly determined and have been previously been shown not to influence competitive ability [17]. All mice were weighed immediately before and after competition. After the first round, males were allowed to recover. During the second competition round, first round winners were place with other first round winners and losers with losers. Because some males were removed prematurely for health reasons from the first competition round, only three males and one female were in each arena for the second round. Mice that had

competed against each other in the first round were excluded from being opponents during the second round of competition.

Competition rounds lasted 3 days. Three morning, three afternoon, and three night observations were made to determine which male occupied the preferred nesting site. Observation times were chosen based on previous experience with this colony and protocol that demonstrated these were resting times. Additionally, wounding was assessed as a secondary measure of high competitive ability. The least wounded mouse was also the majority possessor of the preferred territory for 92% of the competition arenas, as expected if he was the male of the highest competitive ability [e.g., 13, 24]. Possession of a preferred nesting territory was considered evidence of high competitive ability and has been directly linked to male fitness [1, 7-9, 11, 25, 26].

For every round that a male won, he was given one point (e.g., males that won the first round but lost the second were assigned a score of 1).

3.3.7 Data Analysis

Data were analyzed with nonparametric methods where possible to improve robustness of inferences due to the skewed distribution of competitive ability and aggression scores. Correlations were done with a Spearman's rank method. Two separate data sets were analyzed. The first set included all individuals with available data (n= 50 or 49). The second set included only individuals who attacked the intruder (n= 21). The analysis was broken into several different questions: 1) What is the relationship between competitive

ability and aggression, 2) what, if any, factors are controlling aggression, and 3) do our measures of aggression display additive genetic variation?

3.3.8 Competitive Ability vs. Aggression

First, competitive ability was correlated with the total number of- and latency to first- attacks. Individuals who did aggress were also grouped by whether they won the first round. A Mann-Whitney U tests to see if there were differences between the groups in total attacks and the latency to first attack.

Assessing a global relationship between aggression and competitive ability is informative; however, it is less informative than asking whether there is a relationship between aggression and competitive ability amongst the members of one competition arena. Therefore, we assessed the relationship between winning the first round of competition and differences in aggression between opponents. We subtracted an individual's aggression score with his opponents' average to produce an "aggression disparity measure." We then used a t-test with individuals grouped based on winning or losing and a logistic regression to look for significant trends.

Intensity of aggression might not be linked to competitive ability, but whether an individual attacked at all might be. This type of analysis places emphasis on the qualitative aspects of aggression rather than specific measures of intensity. To this end, we used a Fisher's exact test to investigate whether individual who attacked also won the first competition round.

3.3.9 Influences on Aggressive Behavior

The disparity in body masses between the resident and the intruder was quantified by dividing the resident's mass by his opponent's mass. Three measures of aggression were investigated separately: whether an individual aggressed, total attacks, and attack latency. The multivariate analysis included several possible explanatory factors: body mass disparity or resident body mass and intruder body mass, an individual's sire's competitive ability, an individual's competitive ability, litter size, and litter sex ratio. Two models for each measure of aggression were completed. One model used body mass disparity; the other used both the resident's and intruder's body masses. An ordinary least squares regression was used. Number of attacks was log-transformed to improve normality.

3.4 Results

3.4.1 Descriptive Results

Of 50 male mice phenotyped, 21 attacked the standard C57/J6 opponent (42%). The total number of attacks ranged from zero to 44. Latency to first attack ranged from 19 to 420 s. These two measures were significantly, negatively correlated ($\rho = -0.87$, $P << 0.001$). The variation seen is likely due to inherent differences in the focal resident males because there was no suggestion that the opponents influenced the results. Specifically, differences in body mass were not correlated with whether a male attacked or the intensity of aggression observed, see below. In addition, C57/6J mice are known to be a nonaggressive strain.

Of the 50 male mice that completed aggression trials, 49 were assigned a competitive ability based on performance in the competitive trials. Of these 49 males, four were 2-time winners, 18 were 1-time winners, and 27 were 0-time winners.

3.4.2 Competitive Ability vs. Aggression in Resident-Intruder Test

Using the complete data set, competitive ability was not significantly correlated with the total number of attacks (ρ= 0.05, *P*= 0.73; **Figure 3.1**) or with the latency to first attack (ρ= -0.062, *P*= 0.67).

Using the reduced data set, competitive ability was significantly negatively correlated with total attacks (ρ= -0.48, *P*= 0.02; **Figure 3.2**), and positively correlated with latency to first attack (ρ= 0.43, *P*= 0.053) in individuals who actually did attack the intruder in the resident-intruder assay.

Individuals who won the first competition round attacked less than losers of the first round (*P*= 0.05), but there was no difference in the latency to attack between the groups (*P*= 0.14).

We also tested whether the level of aggression an individual displayed in the resident-intruder test against C57/6J was predictive of the outcome of the competition trials against other wild mice. The difference between an individual and the mean intensity of aggression of his opponent did not predict the first-round winners (*P*= 0.75, **Figure 3.3**).

No significant relationship was seen between whether an individual attacked at all and winning the first round of competition; although, there is a suggestion of a trend using Fisher's Exact test (*P*= 0.12).

3.4.3 Influences on Aggressive Behavior

Three separate analyses were run to investigate factors that influenced 1) the decision to attack (yes/no), 2) total number of attacks, and 3) latency to first attack. This analysis was performed with both the complete data set and one that contained only individuals who did attack, where applicable. Each analysis included either the body mass disparity measure or the absolute body masses of both the resident and intruder, an individual's sire's competitive ability, an individual's competitive ability, litter size, and litter sex ratio.

The decision to attack was influenced positively by competitive ability (b= 1.16, *P*= 0.04) and negatively by litter sex ratio (b= -6.32, *P*= 0.02), in the model containing body mass disparity (**Table 3.1**). It was influenced by the same factors in the model containing the absolute body masses of the resident and intruder (**Table 3.1**).

Total attacks were negatively influenced by litter sex ratio (b= -1.3, *P*= 0.03), with the model containing body mass disparity (**Table 3.1**). Litter sex ratio again displayed a negative influence in the model containing the absolute body masses of the resident and intruder (b= -1.4, *P*= 0.02; **Table 3.1**).

Using the reduced data set, no factors were significant in the model containing body mass disparity, but competitive rank displayed a suggestive

trend (b= -0.29, *P*= 0.053). In the model using the absolute body masses, competitive rank was again suggestive (b= -0.27, *P*= 0.077).

Latency was influenced positively by litter sex ratio (b= 304, *P*= 0.03), in the model containing body mass disparity (**Table 3.1**). There was also a positive influence by litter sex ratio in model containing the absolute body masses (b= 311, *P*= 0.03; **Table 3.1**).

Using the reduced data set, no factors were significant or displayed suggestive trends (**Table 3.1**).

3.5 Discussion

This study examined how offensive aggression is related to competitive ability. Aggression displayed no strong overall relationship to competitive ability; however, a subtle link between the decision to attack in the resident-intruder test and competitive ability was found. Aggression did not influence the outcome of competition trials suggesting that the two traits are distinct phenomena (**Figure 3.1, 3.3**). Specifically, differences in aggression between opponents did not predict the winner of a multiday competition in a seminatural arena (**Figure 3.3**). Our results strongly support the suggestion that aggression and competitive ability should be treated as separate variables, especially during a first analysis [27-29]. However, when restricted to those individuals who did show aggression in the resident-intruder test, the intensity of aggression and competitive ability are significantly, negatively correlated; i.e., the most aggressive individuals are of the lowest competitive ability (**Figure 3.2**). Further, multivariate models of the data

showed a weak, positive influence of competition ability on the decision to attach, but not on the intensity of aggression displayed (**Table 3.1**). Interestingly, the difference in body mass between the resident and the intruder did not influence the resident's decision to attack, despite a competitive disadvantage for smaller males [17, 18]. Individuals from male-biased litters were found to be less aggressive (**Table 3.1**). Finally, our study emphasizes the need for evolutionary minded behavioral test and analyses.

Competitive ability and aggression are weakly linked traits using this data set within male house mice. This result is consistent with the only other study to assess aggression's relationship to competitive ability using a separate measure of each trait (**Figure 3.1, 3.2**) [30]. However, this finding is in direct disagreement with most of the work done on laboratory strains of mice [13-15]. It is also inconsistent with a study by Blanchard et al. [31], which using laboratory rats, did link aggression tested before grouping and competitive ability in a seminatural arena. The difference between our study and that of Blanchard and colleagues is likely attributable to aggression *per se* playing a more integral role in the formation of social hierarchies within rats [31, 32]. Blanchard and colleagues [31] also used aggressive acts towards an intruder as the measure of competitive ability. There are also differences between the socioecology of rats and mice that might explain the divergent results [7]. Collectively, there are now many reasons, empirical and theoretical, to ensure that evolutionary minded definitions of aggression and competitive ability have been established before beginning an

experiment. Our results strongly suggest that aggression and competitive ability, while possibly linked, are never synonymous *a priori* [13, 14, 30, 31, 33].

Besides differences in body mass, five additional factors were investigated for potential influences on aggressive behavior; body mass of resident, body mass of intruder, resident's sire's competitive ability, litter sex ratio, and litter size. Aggression was influenced by two factors; competitive ability and litter sex ratio (**Table 3.1**). Most of the observed influences were the same in models containing body mass disparity and the absolute body masses of the resident and intruder; and found using the full data set. Competitive ability positively influenced a resident's decision to attack, meaning that individual of high competitive ability were more likely to attack the intruder (**Table 3.1**). There was a suggestive trend that high competitive ability negatively influenced total attacks using the reduced data set (**Table 3.1**). Both of these effects are suggesting that aggression and competitive ability are distinct phenomena. Litter sex ratio was the most influential factor on aggression overall. Male-biased litter sex ratio negatively influenced the decision to attack, total attacks, and positively influenced the latency to first attack. This result is in conflict with a study designed to investigate this particular phenomenon by Vom Saal et al. [34], which found that males *in utero* between two sisters were less aggressive. Collectively, these results suggest that individuals from male-biased litters are less likely to attack, take longer to attack if they do, and are less intense if they do aggress. It should be noted that although we hypothesize *in utero* effects are the most probable cause driving our observations; our results cannot differentiate

between *in utero* effects, effects from biased litters during postnatal development, and other possible mechanisms. Additionally, our measure of *in utero* hormonal environment is not informative about the position of any individual male, suggesting caution when interpreting these results.

Our data suggest that males of high competitive ability are the most likely to aggress, albeit less intensely than males of low competitive ability (**Table 3.1**). Meaning, as competitive ability increases, so does an individual's willingness to attack. Within an evolutionary context, individuals of high competitive ability are likely to have high quality territories. Therefore, males should assess the threat of every encountered male to compete for their resources. If an intruder is found to be of low ability, then a dominant male might/should back off and not risk injury. Collectively, these data suggest that competitive ability and aggression are linked in a predictable way, although aggression does not seem to be a determinant of competitive ability.

One of the most interesting results is that differences in body mass between the resident and the intruder did not influence the decision of the resident to attack, the frequency of attacks, nor the latency to attack. Additionally, body mass, both the disparity between opponents and the absolute body masses, were not significant in any of the multivariate models (**Table 3.1**). This result is somewhat surprising because relatively small males are at a competitive disadvantage, although it is consistent with a similar study on a laboratory strain of rats [17, 18, 31]. This suggests that males are willing to defend their territory against all opponents regardless of differences in body mass alone. This

conclusion is supported by results of a similar study by Gray et al. [35], who also found that size differences did not affect the intensity or frequency of attack by residents. It also makes sense given the gravity of losing an agonistic conflict. A male's disregard for size disparity seems appropriate when the alternative is loss of resources and thus fitness.

A very interesting question arises from the data: What is purpose of aggression? Our data suggest that individuals who attack quickly and intensely are of low competitive ability. One would predict that individuals of low ability would be timid because they are not likely to win the fights that they start. Perhaps, a male aggressing intensely is advantageous in some circumstances. If two individuals are of equal ability, then one individual signaling through hyperaggression a willingness to "go all in" over a contested resource might decide the outcome. In this way, a highly aggressive nature might be advantageous, especially for individuals of medium to low competitive ability.

A more technical result of this research, along with other studies, empirically emphasizes the need for ecological and evolutionary meaningful tests of behavior [30, 33, 35, 36]. For house mice, this means that five to ten minute encounters in neutral resource-devoid boxes are likely assessing aggression, not competitive ability. Contrived tests that are supposed to assay important life history traits or behaviors, such as competitive ability, that remove test subjects from the evolutionary context of the behavior should be interpreted carefully. The importance of male competition over resources was also elegantly demonstrated by Zielinski and Vandenbergh [36] and Gray et al. [35]. Zielinski and

Vandenbergh [36] manipulated the testosterone (T) to generate high and low testosterone males. When tested over 2 days in standard colony cages, the two classes of males did not differ in competitive ability. However, when pairs were tested in the presence of females, high T males won 11 out of 14 trials. Gray et al. [35] also demonstrated that males attacked more quickly and intensely in the presence of resources. Additionally, if aggressive acts are the basis of scoring competitive ability, then the most aggressive individuals must also be the most competitive. It is important to note that none of the previous studies of house mice, which contradict our results, measured competitive ability and aggression based on different definitions of these behaviors.

This is the first study to demonstrate empirically within a mammal that innate aggression has little influence on competitive ability. In fact, highly aggressive individuals were usually of low competitive ability. However, males of higher competitive ability did decide to become aggressive against an intruder more often, although less intensely, perhaps establishing a link between aggression and competitive ability. Differences in body mass did not predict a resident's decision to attack, suggesting that males defend their 'territory' from all conspecifics. Aggression is suggested to be heritable, which might be advantageous to certain families of intermediate competitive ability. This research suggests caution when interpreting many laboratory tests that remove subjects from the evolutionary context of the focal behavior. Importantly, this study continues to add to the growing literature that suggests a complicated and

highly integrative foundation for competitive ability, an extremely important behavior to most male mammals.

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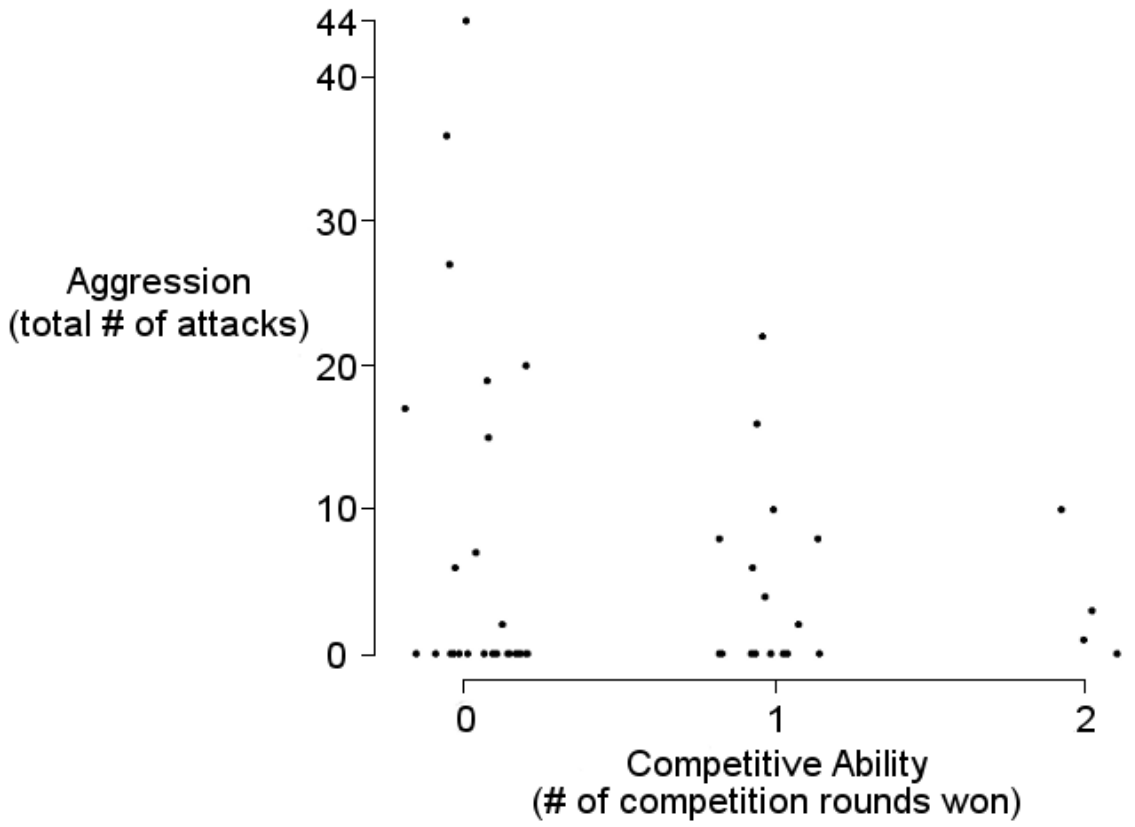

Figure 3.1. Graphical representation of the correlation between competitive ability and aggression seen in a resident-intruder test before competitive trials. Total number of attacks during resident-intruder aggression trials *versus* competitive ability [# of rounds won during a 2 round competition]. The two measures are not correlated [ρ= 0.05, p= 0.73, n= 49]. Random noise was added to the x values to help visualize the data.

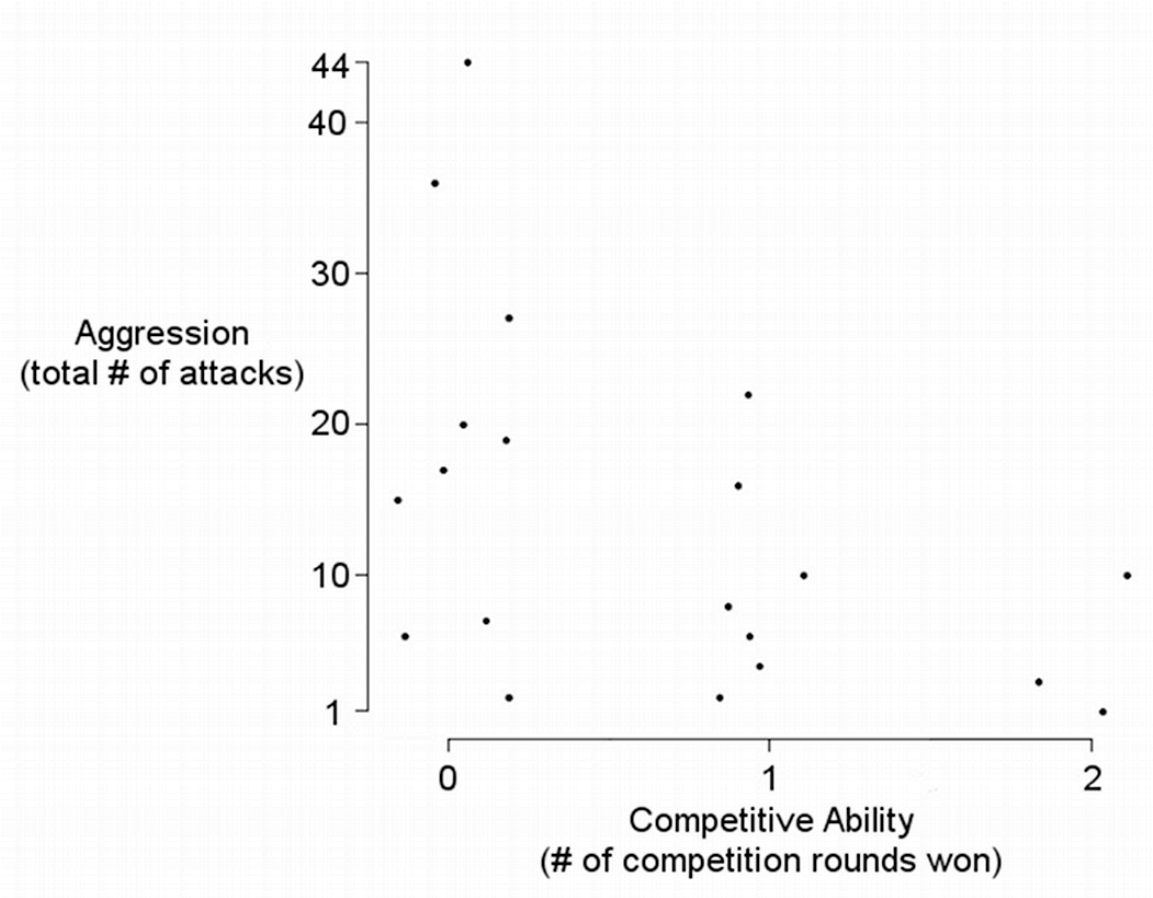

Figure 3.2. Graphical representation of the correlation between competitive ability and aggression restricted to those individuals who showed aggression in a resident-intruder test. Total number of attacks during resident-intruder aggression trials *versus* competitive ability [# of rounds won during a 2 round competition]. Data set is compiled from only those individuals who did aggress. The two measures are negatively correlated [ρ= -0.48, p= 0.02, n= 21]. Random noise was added to the x values to help visualize the data.

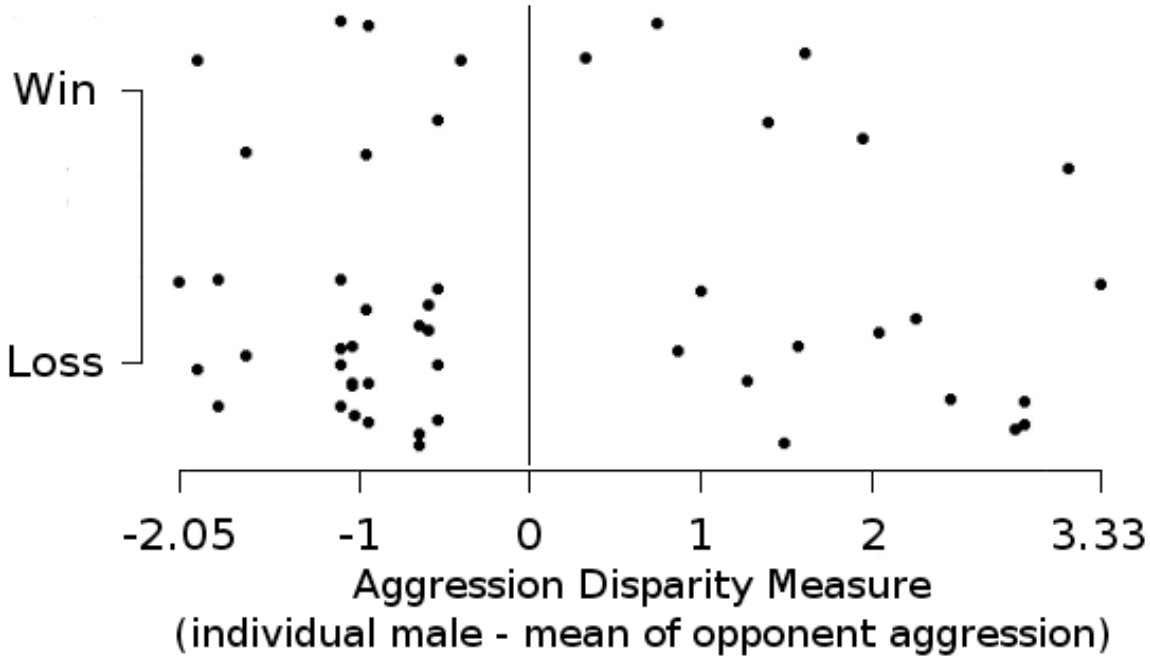

Figure 3.3. Graphical representation of influence of differences in aggression on competitive ability within first round of competition. Win/loss of the first competition round *versus* aggression disparity measure (individual male – mean aggression score of opponent). The level of aggression disparity did not predict which individual became dominant using a logistic regression (p=0.75).Vertical line at 0 represents the value at which the score of the two groups are equal. Random noise was added to the y values to help visualize the data.

CHAPTER 4

MUP EXPRESSION, SOCIAL EXPEREINCE, AND COMPETITIVE ABILITY IN MALE HOUSE MICE (MUS MUSCULUS)

4.1 Abstract

Although success in physical conflict is a major determinant of mammalian fitness, little is known about the role of olfaction and chemical communication in establishing an individual's competitive ability. Mice excrete large amounts of protein in their urine, most of which are Major Urinary Proteins (MUPs). MUPs are polymorphic and are involved in signaling individual identity, and their expression responds to changes in the social environment. However, it is not known how MUPs expression relates to competitive ability before and after physical conflict. Here, we assessed the relationship between MUP expression and competitive ability and cumulative experience in a socially competitive environment. Mixed sex groups were introduced into seminatural enclosures over multiple days to assess the competitive ability of individual males; urine samples were taken before and after each of two rounds of competition. Overall, competitive ability did not show any strong links to MUP expression. However, MUP expression was strongly positively related to the amount of social

experience, supporting the idea that MUPs help mediate social communication. We also identified a link between a sire's competitive ability and his sons' MUP expression, suggesting the possibility that epigenetic mechanisms help regulate MUP expression. Creatinine was used to standardize MUP expression across individuals; normalized MUP expression values were consistent with non-normalized values. Surprisingly, creatinine levels themselves were affected by both social experience and body mass, suggesting care must be taken when using creatinine to standardize MUP expression levels. In conclusion, our study suggests that the social controls regulating MUP expression are complex and dynamic.

4.2 Introduction

Physical conflict is an almost universal behavior among animal species. Hunter [1], and later Darwin [2], were among the first to identify fighting as a major determinant of anatomical, behavioral, and life history traits. Since then, physical conflict has been confirmed to play a significant role in animal evolution [3, 4].

In many animal species, an individual's success in agonistic physical conflicts leads to a monopolization of reproductive resources and greater reproductive success [3, 5-15]. Once established, social behavior networks are often stable over long time periods, sometimes even in the absence of continued physical competition [16], and are a persistent influence on behavioral and mating strategies [9, 16]. While the influence of competitive ability on

reproductive behavior has been studied in many species, less is known about the specific traits that determine and advertise competitive ability, such as social signals. For instance, although pheromones and odorants involved in chemical communication are central to social interactions in animals, the identity, function, and regulation of odorants in relation to competitive ability are poorly understood [17].

In house mice, male fitness is principally determined by competitive ability [3, 11, 12, 15, 18-20]. Males compete for territories, which include limited resources and which attract multiple high quality females [12, 18-21]. Females mate almost exclusively with highly competitive males [15, 21-23] and physical competition forms the basic structure of mouse social groups (i.e., demes) [19, 20, 24]. We have recently completed an experimental assessment of competitive ability in wild-derived mice and demonstrated a striking amount of additive genetic variation in competitive ability, and we identified several associated behavioral and physiological traits [25, 26]. Here, we extend these studies to address the role of chemosignals, specifically Major Urinary Proteins (MUPs), in the advertisement of competitive ability.

Olfaction is a key component of social structure in mouse populations because excreted odorants can advertise social status and territorial ownership [27-29]. Chemical communication is likely to facilitate stability in competitive relationships because territorial ownership is reinforced by urinary marking and countermarking a defined patch of ground [28, 29], with dominant males depositing more scent marks than subordinates [30, 31]. Scent-marking rate in

natal litters predicts certain aspects of adult competitive ability [32]. Consistent with these observations, female mice prefer to mate with a male whose territory only contains his scent marks or a male who reliably countermarks a competitor's scent [28, 29]; furthermore, when given a choice, females prefer the odors of highly competitive males over low competitive males, even in the absence of the males themselves [33]. Scent marks have also been suggested to be costly, and by extension, to honestly advertise health and vigor [34]. Understanding the role of urinary chemosignals might help elucidate some of the mechanisms that bias fitness toward individuals of high competitive ability.

Several lines of evidence suggest that major urinary proteins (MUPs) might play a role in the establishment and advertisement of competitive ability in house mice. Mice excrete large amounts of protein in their urine, nearly all of which (>95%) are MUPs [35, 36]. MUPs are protein pheromones that are encoded by many (>15) linked loci and are an integral part of social communication within this system [37]. MUPs were traditionally viewed as pheromone vectors because they provide a mechanism for the slow time-release of volatile, hydrophobic pheromone ligands that are bound in a protected fold within the mature proteins [38]. More recently, a qualitative role of MUPs has been identified in many important biological functions. For instance, MUPs play a functional role in individual recognition [39], sex recognition, and kin recognition due to their multicopy, polymorphic nature [37]. MUPs also mediate reproduction by signaling estrous cycle stage [40], increasing ova number release under high density housing conditions , and accelerating the onset of puberty (though this

last effect is debated) [41, 42]. The ontogenetic beginning of MUP expression likely triggers the onset of parental aggression, thus promoting the dispersal of maturing males [43]. The quantitative role of MUP expression also appears to have important behavioral functions. MUP expression is sexually dimorphic, with males expressing roughly three to four times that of females [44], and is influenced by genetic [39], epigenetic mechanisms [45], olfactory environment [46], and hormones (testosterone, growth hormone, thyroxin) [47], suggesting a possible role of the social environment in modulating expression [34, 48]. Females have been shown to prefer scent marks with higher MUP concentration [49]. Additionally, MUPs *per se* have been demonstrated to increase aggression in a concentration dependent fashion and the ligands bound to MUPs have also been found to co-vary with competitive ability in laboratory strains, specifically farnesenes [27, 50]. Intriguingly, during competitive scent-marking trials, males most actively countermark a foreign scent mark that only contains the non-volatile fraction of the urine, which is nearly exclusively composed of MUPs [51]. Despite their overwhelming importance to *Mus* biology, how regulation of MUP expression relates to competitive ability has never been investigated directly.

Here, we investigated the possible role of major urinary proteins (MUPs) in the foundation and advertisement of competitive ability by measuring MUP expression before and after repeated rounds of social competition. We addressed three questions regarding the role of MUP expression in male-male competition. First, what is the relationship between competitive ability and MUP expression? MUPs represent an irretrievable loss of protein, and scent marking

behavior has been linked to reduction in body growth and adult body weight, suggesting that scent marking is in a trade-off with somatic growth [34]; thus, one prediction is that individuals in the best condition should be able to invest the most into this costly signal (and thereby succeed in attracting mates). Consistent with this model, Garratt et al. [52] recently demonstrated that MUP and associated ligand expression is depressed as males age, a predicted result if energetically costly signaling declines with senescence [52]. An alternative prediction is that, if MUP expression is decoupled from competitive ability, highly competitive males may conserve energy and resources by limiting excretion of MUPs in the urine.

Second, what is the effect of repeated exposures to male-male social competition on MUP expression? While some studies have examined MUP expression in various social conditions under caged, laboratory settings, less is known about how MUP expression responds to social competition in a more naturalistic setting. Here, social environment is defined as unrestricted interactions with multiple individuals in enclosures with prime and suboptimal nesting sites.

Third, are there transgenerational effects of paternal competitive ability on offspring MUP expression? Our study also investigates the possibility of a transgenerational or nongenetic influence on MUP expression. This was possible because sires of the current test subjects were also measured for competitive ability, and each randomly mated to naïve females that were unassociated with the competition assays [25]. By mating both highly competitive and low

competitive males with randomly assigned mates, this breeding design allowed us to assess if a sire's competitive ability had a transgenerational influence on his offspring. Previous studies have suggested that both paternal [53] and maternal experiences influence certain aspects of competitive ability of sons [54]. In addition, transgenerational responses to differing mating systems (polygyny vs. forces monogamy) have been found to modulate the expression of MUPs [45]. Finally, MUP expression in the liver has been shown to affect transgenerational epigenetic inheritance [55, 56] The functional significance of transgenerational epigenetic modifications to MUP expression remains unknown.

We used an ecologically relevant test of competitive ability--a multiday competition in a seminatural arena with reproductive resources--to assess the relationship between MUP expression and competitive ability. We tested whether MUP expression level was predictive of, or responsive to, the competitive ability before and after each of two competition rounds. These serial time samples were also used as a measure of social experience to assess the long-term relationship between MUPs and sociality. We repeated the analyses with creatinine-controlled MUP expression to help control for the effects of dehydration between subjects. Creatinine itself was also examined to find trends across the study and associations with the social factors. From here on, "MUP output" refers to protein level in urine and "MUP concentration" refers to MUP output normalized with creatinine.

4.3 Methods

4.3.1 Subjects

Members of the 13th generation of an outbred, wild-derived house mice (*Mus musculus*) colony were obtained from the Department of Biology, University of Utah. We used standard breeding and care protocols, see Cunningham et al. [25] for a full description. Individuals were 11 months at testing, which is considered the young adult life stage for wild-derived colonies [52, 57]. The University of Utah IACUC approved all protocols.

4.3.2 Competition Arenas

Competitions were staged in seminatural enclosures made of acrylic (140 x 60 x 15cm). Enclosure design was adapted from the established “phenotron model system” developed by the Potts laboratory [58]. This system is based on the innate tendency of house mice to compete for territories that include dark, isolated nest sites that protect from predators and infanticidal conspecifics and was originally used to study mating preference [21, 22, 24, 59]. Enclosures were divided into 1) a preferred nesting territory and 2) a larger communal area that is a suboptimal territory. The preferred site was dark, contained food and water, and nesting material (paper towels) (15 x 30 x 15cm). The larger area was transparent, had communal food and water, and lacked nesting material. The large communal space provided multiple, small retreats to help males avoid social interactions. A remotely operated, sliding door allowed physical separation

of the preferred and communal areas before researchers entered the testing room.

4.3.3 Preparation Procedures

Competition in each arena was additionally motivated by adding a randomly selected female mouse that was separated into a single cage for 2 weeks before the beginning of the competition. One week before the competition rounds began, passive integrated transponders were implanted between the scapulae of males, which helped to rapidly identify via a handheld receiver and reduce disturbance during testing.

4.3.4 Competitive Ability Testing Procedures

Our competitive ability assay consisted of 2 weeklong rounds of competition. Groupings of mice for the first competition round were randomly generated (excluding siblings, male or female) from lists based on their sire's competitive ability. The father's competitive ability was established by the same protocol described here, which is described in detail elsewhere [25]. In competitive ability assay, we selected one son from a 2-time winning father, two sons from different 1-ime winning fathers, and one son from a 0-winning father. All mice were inspected for conspicuous abnormalities or injuries since the first inspection at weaning. All mice were then weighed, ear punched (for identification), and placed within the arenas. The order of placement into the arena and ear punches were assigned randomly and determined to have no

influence on competitive ability [25]. All mice were weighed again as they were removed from the arenas. After completion of the first round of competition, males were given 6 weeks to recover. During the second competition round, males that won the first round were placed in arenas with other winning males and first round losers were placed with other losers randomly. Mice that appeared under serious stress were removed. Any mice that were removed due to apparent stress were excluded from the second competition round. Because some males were removed, only three males and one female were placed in each arena for the second competition round. Mice that had competed against each other in the first round were excluded from being opponents during the second round of competition.

A competition round lasted 3 days. Three morning, three afternoon, and three night observations were made to identify the holder of the preferred nesting site. Times of observations were based on previous experience with this protocol that showed them to be daily resting periods for this population. Possession of the preferred nesting territory was considered evidence that a male was highly competitive. Male mice were also assessed for quantities of conspicuous superficial bite marks sustained during the competition trials on their tail and back separately. A lack of superficial wounding was used as a secondary measure of competitive ability and correlated with the majority holder of the preferred site in 92% of the arenas [13, 60].

4.3.5 Urine Sampling

MUPs were sampled at four periods. Timepoint one was 1 week before the beginning of the first competition round. Timepoint two began the afternoon that the mice were removed from first competition round. Timepoint three was one week before the beginning of the second competition round. Timepoint four began the afternoon that the mice were removed from the second competition round. This sequential series of urine samples was used as a proxy for time spent in a social environment.

Urine was collected by pipettor and stored in eppendorf tubes placed on dry ice until moved into a -70°C freezer, where they remained until analysis.

4.3.6 MUP Expression Analysis

Following a 1:8 dilution of whole urine, total urinary protein concentration (>95% is MUPs [35, 36]) was determined with the Bradford Assay (Pierce) according to the manufacturer's instructions. Urine was analyzed using a 96-well plate spectrophotometer (Bio-Tek Synergy HT). To reduce technical variation, assays were run with all samples from either the first or second competition round on a single 96-well plate.

4.3.7 Creatinine Analysis

Whole urine was used to measure creatinine using a colorometric assay based on Jaffe's basic picrate method (Stanbio Liquicolor Kit), according to the

manufacturer's instructions. Samples were analyzed on 96-well plates using a spectrophotometer as above.

4.3.8 Statistical Analysis

Data were first converted to standard units. MUP output and creatinine were measured in units of mg/ml; MUP concentration was a ratio of MUP output to creatinine. We realize that the serial time samples we took from the same individuals generally warrants a repeated measures approach. However, our data set is unbalanced and incomplete. This makes a repeated measure analysis approach difficult. For example, with a repeated measures ANOVA, the unbalanced design produced singularities because we only have four two-time winners while trying to estimate the influence of five variables. A generalized linear mixed models approach was also attempted; however, the models were mostly unstable. We have chosen to take a conservative approach with a multiple regression. It should be noted that a regression has less power than a repeated measures analysis due to an inflated error variance compared to a repeated measures analysis. Therefore, our conclusions should represent the most conservative interpretation of our analysis.

Data were then checked for normality using a Shapiro-Wilks test. MUP output was normally distributed (p= 0.28). Creatinine and MUP concentration were $\log_{10}$ transformed; both were normally distributed after the transformation (p= 0.69; p= 0.51; respectively).

Data were first analyzed within a univariate framework. MUP output, creatinine, and MUP concentration were regressed against body mass, body mass change between the beginning and end of a competition round, competitive ability (win or lose first and second round and their interaction), sire's competitive ability, and social experience (sampling time point).

Next, we used a multivariate multiple regression to analyze MUP output, creatinine and, MUP concentration as dependent variables. The full model included an individual's competitive ability (win or lose first and second round and their interaction), his sire's competitive ability, body mass, and timepoint (a proxy for social experience) as factors. A stepwise, backward selection of the best model was preformed for each dependent variable. Model selection was based on corrected-Aikike Information Criterion (AIC_C) scores, which penalizes models for the number of parameters estimated.

All analyses were preformed in R [61].

4.4 Results

4.4.1 Univariate Analysis of MUP Output, Creatinine, and MUP Concentration

All results can be found in **Table 4.1** from the univariate analysis. Only significant results are reported below.

MUP output was positively associated with sampling time point ($b = 0.23$, $p < 0.001$; **Figure 4.1**).

Creatinine was positively associated with body mass (b= 0.011, p= 0.01). Creatinine was negatively related with sampling time point (b= -0.058, p < 0.001). See **Figure 4.2**.

MUP concentration was negatively associated with body mass (b= -0.011, p= 0.008). MUP concentration was positively associated with sampling time point (b= 0.084, p < 0.001). See **Figure 4.2**.

4.4.2 Multivariate Analysis of MUP Output, Creatinine, and MUP Concentration

All analyses originally included whether an individual won the first round, the second round, the interaction between the two round outcomes, his sire's competitive ability, body mass, and time point (**Table 4.2**).

The original full model best modeled MUP output. Whether an individual won the first round, body mass, and social experience had significant partial regression coefficients (b= 0.53, p= 0.015; b= 0.05, p= 0.03; b= 0.25, $p < 0.001$, respectively).

Creatinine was best modeled by body mass and social experience (ΔAIC_C= 34.9). Only sampling time point had a significant partial regression coefficient (b= -0.05, $p < 0.001$).

MUP concentration was best modeled by whether an individual won the first round, his sire's competitive ability, and social experience (ΔAIC_C= 53.5). Both sire competitive ability and time point had significant partial regression coefficients (b= -0.04, p= 0.04; b= 0.08, $p < 0.001$, respectively).

4.5 Discussion

This study investigated the regulation of Major Urinary Protein (MUP) pheromones in relation to competitive ability—the primary determinant of male reproductive success in mice. Urine samples were taken before and after each of two competition rounds in seminatural enclosures specifically designed to assess male competitive ability. The main conclusion of the study is that males continually up-regulate MUPs as they are exposed to a social environment (**Figure 4.1, 4.2; Table 4.1, 4.2**). Interestingly, no consistent overall effect of competitive ability was detected (**Figure 4.1**). However, winning the first competition round had a small effect that increased an individual's MUP output. MUP concentration was significantly, negatively related to body mass across the entire study (**Table 4.2**). A negative association with an individual's sire's competitive ability was also detected (**Table 4.2**). Creatinine was found to be positively associated with body mass but negatively associated with social experience. Collectively, these results suggest that social control of MUPs is complex and highly dynamic.

Social experience exerted the greatest influence on MUP expression (**Figure 4.1, 4.2; Table 4.1, 4.2**). Results from the univariate and multivariate statistical models showed a consistent and strong effect of accumulating social experience and increased MUP output and MUP concentration. This result is consistent with other studies that demonstrated up-regulation of MUPs in the presence of other individuals and novel olfactory cues [44, 46]. Overall, MUP output increased 23% over the course of the study, although it is likely that this is

an asymptotic function. MUP expression has previously been shown to change dynamically with age; while MUP expression increases dramatically during puberty [43], middle-aged male mice show a gradual decrease over six months in expression that continues with senescence [52]. Males in this study were around 11 months of age when the experiment began, and therefore would be predicted to decrease MUP expression if social experience was not an important regulatory mechanism. Additionally, males of this population exposed to a similar social experience in seminatural conditions had higher MUP expression than age-matched male siblings isolated in colony cages (Nelson unpublished data). Our finding that MUP expression increases with increasing social experience is consistent with the established idea that the quantity of MUPs plays an integral role in social communication.

It was difficult to predict how MUP expression would relate to competitive ability *a priori*. Highly competitive males might increase expression to conserve resources, or, they might increase expression to advertise competitive ability. Alternatively, MUP expression may indirectly respond to other cues, such as testosterone [47], that are directly modulated by social experiences. Our study did not find a consistent trend between competitive ability and MUP expression (**Figure 4.1, 4.3; Table 4.1, 4.2**). Nevertheless, the multivariate analysis showed a weak positive effect of winning the first round of competition on MUP output only (**Table 4.2**). This indicates that males of at least moderate competitive ability had greater urinary MUP output relative to males of low competitive ability across the study. However, this effect was not retained in the MUP concentration model.

Collectively, our results do not provide a strong link between competitive ability and MUP expression.

Taken together, these results suggest that while MUP expression responds to the process of social competition, the overall urinary concentration of MUPs does not convey information about competitive ability *per se*. There are several possible explanations for this result. One is that MUPs may not be important in conveying information on competitive ability. It seems unlikely, however, that this highly abundant olfactory signal would not impart information on such an important trait to house mice [15, 17]. Alternatively, it may be that MUPs *per se* do not convey information on social rank, but rather it is some bound ligand that conveys this information [27]. It is also important to note that dominant males scent mark at a much higher rate than subordinates [30, 31]. This means that highly competitive males might have greater absolute expenditure of energy on MUP expression, even if they do have comparable MUP expression to low competitive males. Similarly, simply depositing a urine mark, with MUPs signaling genetic identity, could be enough to convey information on competitive ability [28, 29]. Scent mark concentration might therefore be less important than scent mark presence or absence [28]. It is possible with a larger study and more complete sampling that a consistent trend would emerge. It would also be interesting to measure the expression of individual MUPs, such as Darcin, that are male-specific and preferentially bind androgen dependent ligands [62, 63].

To date, one other study has examined the relationship between competitive ability and MUP expression [46]. This study allowed males visual and olfactory access to conspecific males through wire mesh that divided an enclosure. From this interaction, males were assumed to have established social ranks. Following this, a 5 minute interaction in a neutral arena was used to assess the social hierarchy males had established. This study concluded that males of higher competitive ability (defined using asymmetry in aggression) had greater MUP expression. However, we suggest two caveats to this interpretation [46]. First, the study did not differentiate between aggression and competitive ability, which are distinct behavioral phenomena, including in this species [26]. For instance, we found no relationship between resident-intruder aggression and competitive ability during a multiday test of competitive ability in the presence of resources, including females [26]. Second, the male-male competition was not over resources, which removes males from the evolutionary context of the behavior. This methodological critique is corroborated by the experiment of Zielinski and Vandenbergh [64], which found no difference in competitive ability in males manipulated for high or low testosterone, but found that high testosterone males won the vast majority (79%) of encounters when a female (reproductive resource) was present. Also, Gray et al. [65] demonstrated that males more vigorously defend areas with females present. Thus, although competitive ability is a robust behavior, it is different from aggression *per se*, and is best determined in naturalistic social environments. Finally, our results

contradict the finding of Janotova and Stopka [46] because we found no relationship between competitive ability and MUP expression.

Body mass was found to affect MUP expression in three ways. First, bivariate regression analysis showed that heavier mice had lower MUP concentration than lighter mice (**Figure 4.2**). Second, our univariate analysis found a significant, negative effect of body mass on MUP output (**Table 4.1**). Third, our multivariate analysis of MUP output found a significant effect of body mass; however, it was a positive relationship between MUPs and body mass (**Table 4.2**). Together, these data provide partial support for the hypothesis that MUP expression is costly, which predicts that increased investment in excretion of these signaling proteins comes at the expense of protein-intensive metabolic functions.

Paternal competitive ability had a significant impact on MUP concentration (**Table 4.2**). By virtue of the breeding design in this experiment, this finding suggests a role of transgenerational or nongenetic mechanisms on MUP expression. Paternal competitive ability of subjects in this study was determined with the same protocol described here [25]. After two rounds of competition, all of the sires were mated to naïve females, thereby eliminating genetic selection on socially dominant males. Our multivariate analysis of MUP concentration showed a negative effect of paternal competitive ability, whereby more competitive sires had sons with lower MUP concentration. This would not be the first time a transgenerational effect on MUP expression has been found due to social competition. Work by Nelson et al. [45] has recently demonstrated that DNA

methylation of the *MUP* promoter is heritable and influenced by the social experiences of the parental generations, and that offspring of promiscuous mating systems have higher MUP expression than offspring from monogamous mating systems. This finding makes some sense in light of several other studies. Cunningham et al. [25] recently demonstrated that competitive ability is heritable. Our male mice were housed individually since weaning, so it is possible they deemed themselves “territorial” and highly competitive [reference]. Thus, because highly competitive fathers sire highly competitive sons, it is possible that competitive sons have little incentive to invest in MUP expression. A qualitative examination of the results gives some support for this hypothesis: 2-time winning sons had the lowest MUP concentration across the entire study.

Urinary MUP concentration depends on the dilution of the urine [44, 46]. Creatinine is used as a indicator of urinary dilution in many MUP studies [e.g., 44, 46]. However, creatinine can only be used to indicate hydration level if every individual is excreting creatinine at an equal rate. This implicit assumption is probably violated in many contexts, especially in a true social context. Changes in total body muscle mass, exercise, and emotional stress all influence the amount of creatinine that is excreted [66]. Creatinine was also found to be negatively associated with age in a recent study of MUP expression and senescence [52]. We preformed the same statistical analysis on creatinine as with MUP output to test directly the suitability of creatinine to standardize MUP expression across individuals. Body mass was positively associated with creatinine, which is expected because creatinine level is linked to muscle mass

(**Figure 4.2; Table 4.1, 4.2**). Surprisingly, results from the univariate and multivariate analyses showed that creatinine was negatively related to social experience, which is a much more difficult trend to explain (**Figure 4.2; Table 4.1, 4.2**). Interestingly, there was no significant relationship between creatinine and body mass change between the beginning and ending of a competition round. While the effects of the social environment may not have had a large influence on MUP expression in previous studies, it is something that will need to be addressed as experiments move into more naturalistic environments over longer periods of time. Collectively, these finding suggest that both body mass and the serial nature of samples, if applicable, should be accounted for in statistical analyses.

In conclusion, we investigated MUP expression in relation to competitive ability and length of exposure to a social environment. No overall relationship between competitive ability and MUP expression was detected. Rather, a relationship between an individual's sire's competitive ability and MUP concentration was found. This suggests the possibility of some epigenetic control of MUP expression. Amount of social experience, as measured by our serial time samples, exerted the greatest influence on MUP expression. This trend is consistent with the suggestion that MUPs are an integral signaling mechanism in social communication. It would be interesting to disassociate MUPs from bound ligands to see how volatile compounds are related to competitive ability and how they change after winning or losing social encounters. Finally, creatinine was positively related to body mass and negatively to social experience. This result

cautions authors to take care when using creatinine to standardize MUP expression across subjects. Collectively, our results point to complex network of factors influencing the production of MUPs.

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Table 4.1. Univariate Analysis of MUP Output, Creatinine, and MUP Concentration.

	Estimate	*p*-value
MUP Output		
Body Mass	0.015	0.45
Body Mass Change	2.491	0.22
Sire CA	0.013	0.91
Social Experience	0.229	< 0.001
Win 1st Round	0.534	0.016
Win 2nd Round	0.132	0.52
Interaction: WIn1 & Win2	-0.538	0.15
Creatinine		
Body Mass	0.011	0.01
Body Mass Change	-0.6	0.2
Sire CA	0.019	0.42
Social Experience	-0.058	< 0.001
Win 1st Round	0.017	0.71
Win 2nd Round	0.056	0.2
Interaction: WIn1 & Win2	-0.063	0.15
MUP Con.		
Body Mass	-0.011	0.008
Body Mass Change	0.774	0.045
Sire CA	-0.027	0.25
Social Experience	0.084	< 0.001
Win 1st Round	0.038	0.4
Win 2nd Round	-0.029	0.49
Interaction: WIn1 & Win2	-0.013	0.86

Bivariate regressions were completed for competitive ability (win or lose first and second round and their interaction), body mass, an individual's sire's competitive ability, and social experience. CA= Competitive Ability.

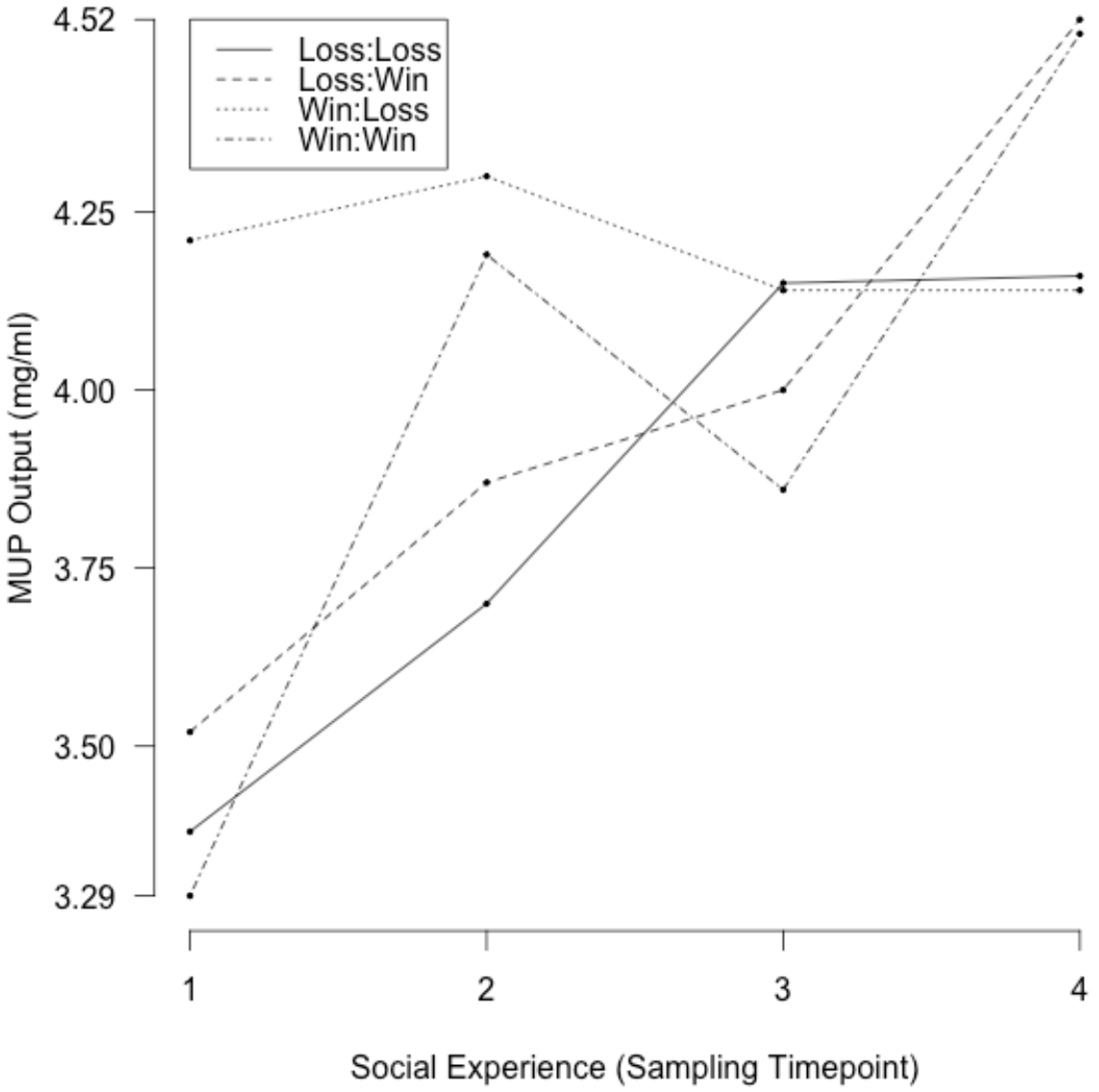

Figure 4.1. Graphical representation of MUP expression over time and in relation to competitive ability. MUP output *versus* social experience (timepoint used as proxy), with competitive ability as an interaction. Social experience was significantly associated with MUP output (b= 0.23, p= <0.001).

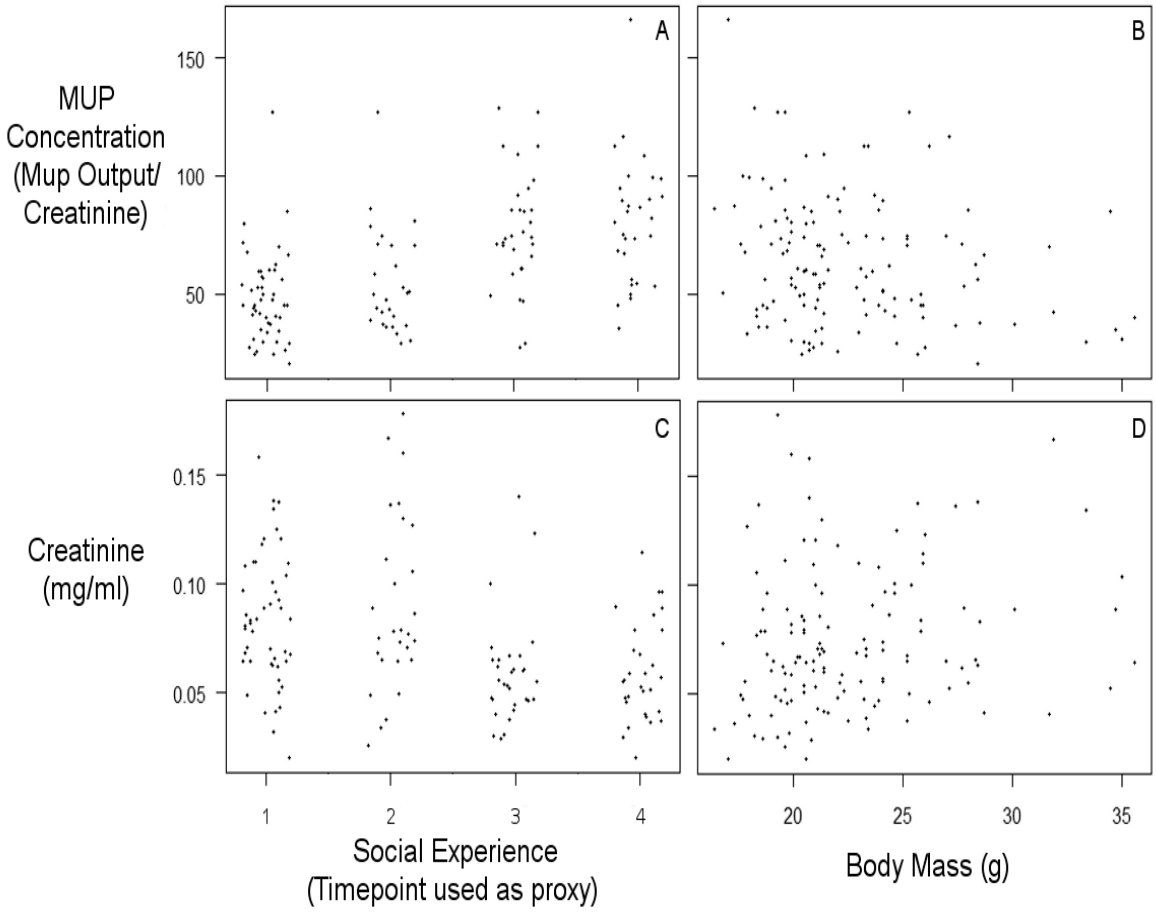

Figure 4.2. Graphical representation of the pairwise relationship between MUP concentration, creatinine, social experience, and body mass. A. MUP concentration *versus* social experience (b= 0.084, $p< 0.001$). B. MUP concentration *versus* body mass (b= -0.11, $p= 0.008$). C. Creatinine *versus* social experience (b= -0.06, $p< 0.001$). D. Creatinine *versus* body mass (b= 0.011, $p= 0.01$).

Table 4.2. Multivariate Analysis of MUP Output, Creatinine, and MUP Concentration.

Full Model					
		Estimate	Std. Error	*p*-value	r^2
MUP Output	Intercept	**2.3**	**0.56**	**< 0.001**	0.14
	Win 1st Round	**0.53**	**0.21**	**0.015**	
	Win 2nd Round	-0.07	0.21	0.72	
	Sire CA	-0.13	0.12	0.3	
	Body Mass	**0.05**	**0.02**	**0.033**	
	Social Exper.	**0.25**	**0.07**	**< 0.001**	
	Interaction: Win1 & Win2	-0.4	0.37	0.28	
Creatinine	Intercept	**-1.2**	**0.12**	**< 0.001**	0.14
	Win 1st Round	2.00E-04	0.05	0.99	
	Win 2nd Round	0.04	0.007	0.33	
	Sire CA	0.02	0.004	0.41	
	Body Mass	0.005	0.005	0.27	
	Social Exper.	**-0.05**	**0.01**	**< 0.001**	
	Interaction: Win1 & Win2	-0.06	0.08	0.42	
MUP Con.	Intercept	**1.6**	**0.11**	**< 0.001**	0.3
	Win 1st Round	0.06	0.04	0.16	
	Win 2nd Round	-0.03	0.04	0.39	
	Sire CA	-0.04	0.02	0.096	
	Body Mass	-0.002	0.004	0.69	
	Social Exper.	**0.08**	**0.01**	**< 0.001**	
	Interaction: Win1 & Win2	0.004	0.07	0.96	

Table 4.2. Continued

Best Model							
		Estimate	Std. Error	*p*-value	r^2	AIC_C	ΔAIC_C
MUP Output	Intercept	Same as Full Model			*	-25.7	*
	Win 1st Round						
	Win 2nd Round						
	Sire CA						
	Body Mass						
	Social Exper.						
	Interaction: Win1,Win2						
Creatinine	Intercept	**-1.2**	**0.1**	**< 0.001**	0.15	-455.7	34.9
	Win 1st Round	*	*	*			
	Win 2nd Round	*	*	*			
	Sire CA	*	*	*			
	Body Mass	0.007	0.004	0.088			
	Social Exper.	**-0.05**	**0.01**	**< 0.001**			
	Interaction: Win1,Win2	*	*	*			
MUP Con.	Intercept	**1.59**	**0.03**	**< 0.001**	0.31	-506.1	53.5
	Win 1st Round	0.06	0.03	0.067			
	Win 2nd Round	*	*	*			
	Sire CA	**-0.04**	**0.02**	**0.043**			
	Body Mass	*	*	*			
	Social Exper.	**0.08**	**0.01**	**< 0.001**			
	Interaction: Win1,Win2	*	*	*			

Models originally included competitive ability (win or lose first and second round and their interaction), body mass, an individual's sire's competitive ability, and social experience in one model. A stepwise, backward selection based on AIC_C scores was used to choose the best-fit model. CA= Competitive Ability.

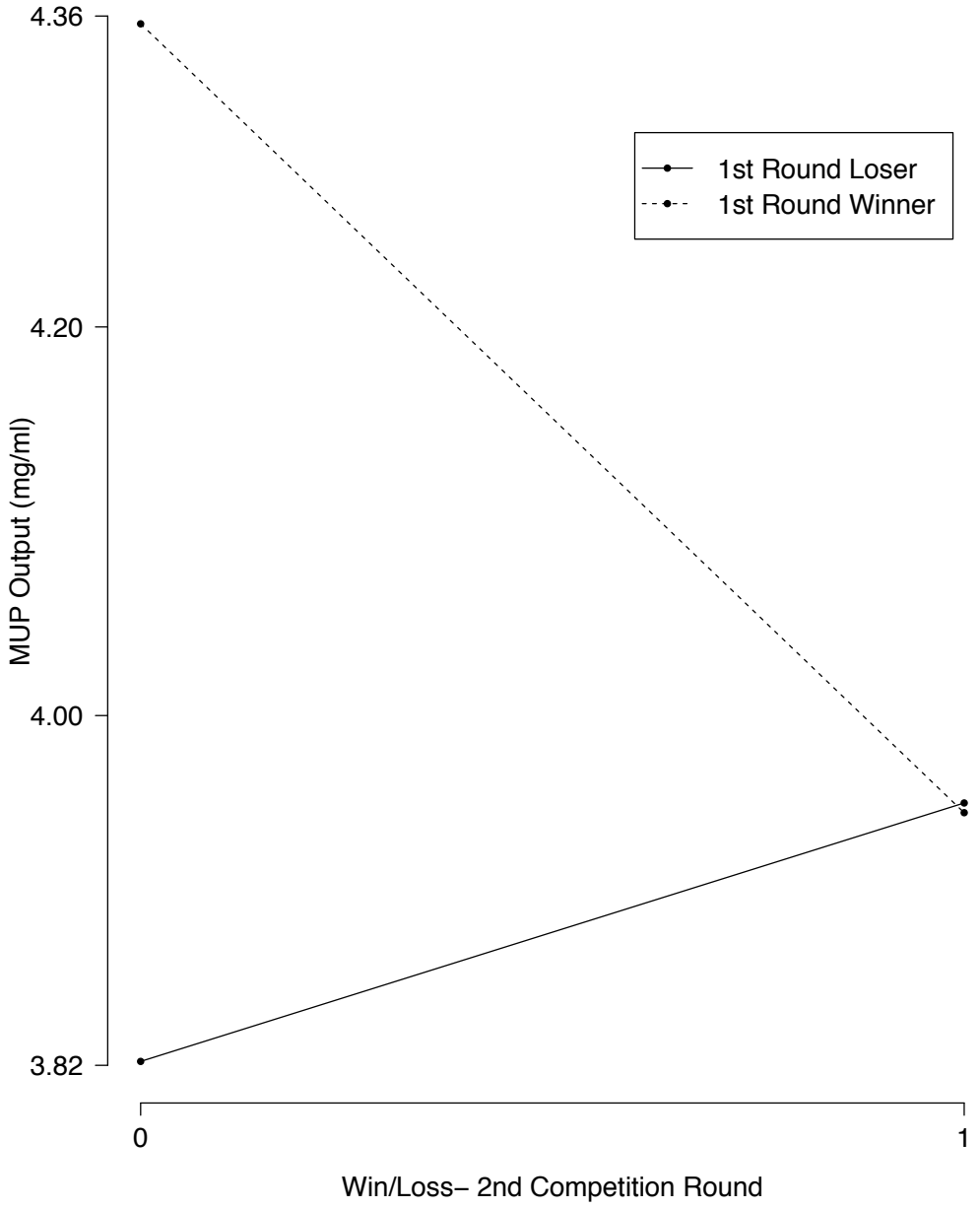

Figure 4.3. Graphical representation of the relationship between MUP output, win/loss in the first competition round, and win/loss in the second competition round. An interaction plot of MUP output *versus* win/loss in the second round of competition with win/loss in the first round as an interaction. Winners of the first round had significantly higher MUP output across the study (slope= 0.53, p= 0.015; **Table 4.1**).

CHAPTER 5

THE INFLUENCE OF MALE-MALE COMPETITION ON PRIMATE BRAIN SIZE

5.1 Abstract

Primates have relatively large brains. Because of its importance, primate neural evolution has been studied extensively, and many factors are correlated with brain size. However the role physical male-male competition may have played in the evolution of primate brain size has yet to be addressed in a broad taxonomic comparison. Physical conflict should entail extensive cognitive interactions, including perception, assessment, reasoning, and neuromotor coordination. Based on this expectation, we hypothesized that primate species that exhibit higher levels of physical male-male competition will have relatively larger brains. To address this hypothesis, we examined the relationship between brain size and two reliable indicators of male-male competition intensity: body mass- and canine height-sexual dimorphism. Analyses were conducted with phylogenetic generalized least squares, using two types of phylogenetic corrections. Significant, positive relationships exist between female brain size and size sexual dimorphism within all of the phylogenetic groupings. No significant relationships were detected between male brain size and indices of

male-male competition. In conclusion, female brain size is positively related with male-male competition overall; however, there is some variability within specific phylogenetic subgroupings. These relationships are consistent with the hypothesis that sexual selection pressures due to physical competition among males are partially responsible for the large brains of primates.

5.2 Introduction

Darwin was the first to grasp the evolutionary significance of physical competition by males for reproductive resources [1]. Primates are a taxon in which male-male competition is thought to be particularly important to the evolution of life history, behavior, and anatomical traits [2-7]. Physical competition's importance has been established by hundreds of studies in both male and female primates by linking social dominance with reproductive success. Ellis [8] reviewed 485 studies that investigated the link between reproductive success and dominance rank among primates. He found that 70% (340) of these studies concluded that a positive link existed between dominance rank and reproductive success, while < 5% found a negative relationship. Among primates, intensity of physical male-male competition is correlated with many traits, including size sexual dimorphism, canine sexual dimorphism, relative male maxillary canine height, hindlimb length, mating strategy, and female reproductive rates [9-15]. Within humans male-male competition has been casually linked to many types violence, including domestic violence and homicide [16]. These empirical correlations and strong theoretical selection pressures [17]

suggest that male-male competition has influenced many traits closely related to fitness within primates.

Primates have also evolved relatively large brains and complex cognitive abilities, which distinguish them from other mammalian orders [18-20]. Because of its importance, primate neural evolution has been studied extensively, and many factors are correlated with brain size. For example, female body mass, percentage of fruits and seeds in a diet, home range size, and social group size are all correlated with brain size [19, 21-24]. Primate brain size has also been correlated with the innovation of novel solutions to repeated ecological and social challenges [18] and ecological success when a species in general is introduced into a completely novel habitat [25]. Surprisingly, the influence of physical male-male competition on the primate brain is not well studied compared to other ecological and social variables. Three studies have investigated the association between physical competition and brain size in primates [26-28]. However, none of these were completed with taxon-wide data for an assortment of reasons.

Physical male-male competition should entail extensive neuromotor integration, observation, assessment, and reasoning. Because the rewards and hazards of physical conflict are high, demands on brain function could select for improved cognitive and neuromotor abilities. The need for precise body movement and accurate opponent manipulation should increase demand on neural motor centers, such as the motor cortex, cerebellum and brain stem [26]. The costs from conflict should also force opponents to critically observe, assess a rival, and predict their rival's actions. This strategic evaluation allows a male to

attack weaker opponents and retreat from stronger ones [29, 30]. Other cognitive tasks necessary for successful fighting could include learning, concept of the future, social ties and coalition building, and awareness of self-condition. While these theoretical selection pressures should have more impact in some neural areas than others, it is likely that the wide-spread distribution of affected areas could lead to an overall expansion of brain size [22]. Indeed, a recent model for the evolution of the largest primate brain, that of *Homo sapiens*, posits a release from direct ecological constraints coupled with large selection pressures due to competition with conspecifics [31, 32]. Humans seem to be an extreme example of the role cognition plays in intraspecific competition. Thus, both the cognitive and neuromotor aspects of physical male-male competition may have contributed to the evolution of large brains in primates.

Alternatively, there are reasons to suspect that physical male-male competition may not be important to the evolution of the primate brain. Factors such as physical prowess alone, social intelligence, coalition formation, ecological pressures, and female choice may present greater selection pressures than the neural aspects of physical competition. It is possible that “strength” (muscle mass, mechanical advantage, etc.) can account for the competitive success of an individual with only minimum neural coordination required. Social manipulation and coalition formation present complex social puzzles involving learning, memory, and strategy. These might be more cognitively demanding and present greater selective pressures than physical competition [33]. Indeed, there is evidence that social factors play an important role in reproductive success in

primate species [8]. Female choice may also present a counter-selective force against males who are physically dominant because females may not choose males based on their ability to physically compete [34]. If females choose less dominant males then any pressure from physical competition might be greatly mitigated, because males are forced to compete in different ways [34]. Physical competition might not be important enough for its impact on the primate brain to be easily detectable given the noise that is almost certainly present from measurement error, etc. If any of these factors are of primary importance to primates as a whole, no relationship will be seen between relative brain size and our measures of competition intensity.

Any evolutionary pressure to increase brain size due to sexual selection has the possibility of influencing one sex more than the other. In our study we would predict that males rather than females would have relatively larger brains due to male-male competition because males are the sex that selection is most likely acting on for this particular pressure [35]. The data set of Isler et al. [36] presents an opportunity to analyze this prediction because it contains data on both male and female primate brain sizes separately. To this end, we estimated the influence of male-male competition within the sexes separately.

To test the hypothesis that the cognitive and neuromotor aspects of physical male-male competition helped select for large brains in primates, we examined the relationship between brain size (BS) and two reliable indicators of physical male-male competition intensity across primate species: size sexual dimorphism (SSD) and maxillary canine height sexual dimorphism (CSD) [10, 11,

14, 37, 38]. We expect that BS would be relatively larger in highly dimorphic species due to the cognitive and physical demands of male-male competition. Regression analyses were performed using phylogenetic generalized least squares (PGLS) to test for significant trends between BS and male-male competition across species. Additional factors known to influence primate brain size, such as sociality and diet type, were not included as covariates because of a lack of order-wide data. Including all known covariates into this analysis would have restricted the analysis to a fraction of the current sample and undermined the premise of a taxon wide test. Covariates were also excluded to ensure the results would be as simple as possible to interpret during this first, order-wide preliminary analysis. It should also be noted that the current hypothesis is not mutually exclusive of other hypotheses about primate brain evolution (i.e., social brain hypothesis), but rather is complementary to them.

5.3 Methods

Species specific values for male and female body mass (grams) and male and female brain size (grams) were taken from Isler et al. [36]. Some species were removed due to low sample size. Male and female maxillary canine heights were compiled from the literature. The phylogeny of Isler et al. [36], which is primarily based on Bininda-Emonds et al. [39], was also used. All data were $\log_{10}$ transformed before any analysis was preformed. One hundred sixty two species were used in the mass dimorphism analyses. One hundred two species were used in the canine dimorphism analyses. Male and female brain sizes were

analyzed separately. Size- and canine-sexual dimorphism (SSD and CSD) was calculated as a ratio: male value/female value.

To control for the effect of phylogeny, species values and dimorphism ratios (male/female) were imported into the APE package of R for Phylogenetic Generalized Least Squares (PGLS) analysis [40]. Two residual error structures were generated and used; a Brownian motion based variance-covariance structure and a correlation based on the maximum likelihood estimate of Pagel's lambda. Papers describing in detail the behavior and advantages/disadvantages of each can be found elsewhere [e.g., 41, 42]. Briefly, a residual error structure based on Brownian motion alone along a phylogeny is equivalent to Phylogenetic Independent Contrasts and a Pagel's lambda value of one [42]. Pagel's lambda scales the off-diagonal elements of the phylogenetic variance-covariance matrix to generate a maximum likelihood correlation structure given the observed data. Only one model/sex/phylogenetic grouping is presented for simplicity of interpretation; the complete results of the analysis can be found in the supplemental material. Model selection was based on corrected Aikike's Information Criterion (AIC_C) scores. In all cases a full complement of models was tested in all combinations; male and female; Brownian motion and Pagel's lambda; SSD, SSD + CSD, and CSD. All analyses included body mass as a covariate to control for allometric trends.

The analyses were run separately for Haplorhini, New World monkeys, Old World monkeys, and Hominoids (Gibbons & Great Apes). While SSD and CSD have not been strongly linked to male-male competition intensity within

Strepsirhines, an analysis was conducted to see if a pattern between BS and indicators of male-male competition intensity could be discerned. Strepsirhines and Haplorhines were also run collectively to see if there was a pattern across all primates.

Because we sampled repeatedly from a population, a Bonferroni correction was used to adjust significance level of the partial regression coefficients of the phylogenetic analyses. This study considered all species together as a single sampling population for the Bonferroni correction. This correction ensures that the interpretation represent a very conservative assessment of the results.

5.4 Results

In each phylogenetic analysis, there was a significant association between brain and body size, as expected (see **Table 5.1**). Interestingly, after the Bonferroni correction, only female brain size had significant associations with competition indices, none of the male phylogenetic groupings did after the Bonferroni correction. The specific female phylogenetic groupings that produced significant association were all primates, Haplorhines, and Old World Monkeys. All of these were positive associations. In all of these models, Pagel's lambda correction was the best-fit model. Overall, out of 12 best-fit models, Pagel's lambda had the lowest AIC_C in 7. All of the 12 best-fit models only included body mass and size sexual dimorphism; none included canine sexual dimorphism.

5.5 Discussion

Because agonistic encounters are likely to be cognitively demanding tasks, we predicted positive relationships between indices of physical male-male competition intensity and brain size among primates. We analyzed female and male brain size separately with the prediction that there would be a greater influence on male brains than female brains from competition intensity. Across every group, there was a positive relationship between female brain size and size sexual dimorphism, which is consistent with the hypothesis. Three of these groups; All Primates, Haplorhines, and Old World Monkeys; had significant associations after a strict Bonferroni correction. There was no consistent trend when using male brain size and only associations with body size were significant after the Bonferroni correction. While not extremely strong evidence, the female results are consistent with the hypothesis that male-male competition intensity played a role in the evolution of large brains in primates.

The models reported were selected based on AIC_C scores (**Table 5.1**). In every case the model containing only size sexual dimorphism (SSD) was the best fit over models containing sexual size dimorphism and canine sexual dimorphism (CSD) or canine size dimorphism alone. This is most likely a function of diminished sample size within analyses including canine size.

One of the most interesting results to emerge from the current analysis is the stronger association between physical competition intensity and female brain size rather than male brain size. We suggest two possible explanations. One is that females are under selection for increased social intelligence. Males of

species that compete intensely are generally much larger than their female counterparts. If brain size increases at a lower rate than body mass, then males will have relatively smaller brain sizes. In support of the first explanation, Lindenfors et al. [26] found that female group size strongly, positively influenced cerebrum size, especially neocortex size. As a general assertion, the many of the most dimorphic species are species that tend to hold harems. This might then place pressure on females to manipulate other group members, while single males would have no such goals. The second explanation is based on allometric relationships. This explanation also has some support. Current evidence suggests that sexual dimorphism evolves by one sex pushing both sexes in one direction [9, 43, 44]. In our case, male-male competition increases brain size and body size in both sexes. Following this initial event, females evolve mechanisms to decouple the genomes and partially return it to a purely natural selection optimum; i.e., females reduce body size closer to a value based on natural selection alone. If they kept most of the newly evolved brain size, then males would have relatively small brains compared to females. Alternatively, males might gain body size to compete better, while brain size stays relatively the same producing females with relatively larger brains as females reduce body mass back towards a natural selection optimum. If one of these situations is true, then brain dimorphism should be less between the sexes than body size dimorphism. This is in fact what we see; brain size sexual dimorphism mean- 0.027, body size sexual dimorphism mean- 0.086. It is also possible that there is some combination of these factors helping to produce the observed trend.

Some work on the effect of male-male competition on brain size has been done previously [26, 27]. This study's results do not support the conclusions by Schillaci [27] in a similar analysis. That study found a negative correlation between brain size residuals and mass dimorphism. Potential causes for the difference are Schillaci's [27] use of much smaller sample size, data from a dated sources, low phylogenetic representation, lack of correction for allometric trends, and general lack of the use of phylogenetic informed analyses. The current study's results are consistent with some of the results of Lindenfors et al. [26], who found that competition positively correlated with the size of aggression and some of the motor centers but negatively correlate with the size of the neocortex. While the study by Lindenfors et al. [26] did not look at overall BS, it did demonstrate that physical male-male competition can be correlated with primate neural evolution.

We would also like to note that our results represent a conservative assessment of the analyses. Very few authors use Bonferroni corrections and some authors have suggested relaxing the α-value to 0.10 given the complicated, integrated nature of the brain [26].

Interpretation of the results of this study is confounded by many factors. These factors include the different phylogenetic groupings with differing selection pressures, non male-male competition that primates engage in, differing importance of male-male competition to different groups, cognitive intensive tasks not associated with male-male competition, and the use of different measures of competition and their associated constraints. All of these factors

could influence these analyses, but were excluded for simplicity and interpretability of results. We feel this concession is justified because this study represents the first order-wide analysis of the impact of male-male competition on the primate brain.

The results generated with Strepsirrhine values, including the all primates grouping, have one caveat. Strepsirrhine SSD, CSD, and relative male canine height have not been strongly correlated with indices of male-male competition intensity (i.e. mating system) [10, 11, 14, 37, 45]. Nevertheless, SSD and CSD accurately reflect physical male-male competition intensity of other taxa; including herbivorous lizards, pinnipeds, shore birds, and carnivores; among others [9, 46-48]. There is also limited evidence that Strepsirrhines do conform to the prediction of sexual selection based on physical male-male competition. Kappeler [45] found that monogamous Strepsirrhine species had less canine sexual dimorphism than polygynous species, although the trend was not significant. There is also evidence to suggest that some lemur species, not previously thought to express body mass sexual dimorphism, do express the predicted dimorphism when using wild rather than captive data [49]. Thus, it is possible that the studies that looked for correlations of SSD and CSD with male-male competition in Strepsirrhines lack power and/or accurate enough data to discern a pattern [14, 37, 45].

Male intrasexual competition in primates is the most likely driver of any association between physical competition intensity and brain size; however, primates engage in many forms of physical conflict that are not restricted to

combat between two males. For instance, some species of Old World Monkeys, which are commonly found in savanna areas, have larger male canines than expected [37]. It is thought that these larger canines might serve for predator defense [37]. Additionally, coalitions are important to some male's success during fights for social dominance; e.g., *Pan troglodytes* and *Brachyteles* [3, 37, 50]. Coalition formation might demand high cognitive capacity but lower physical prowess because any single individual's physical ability is less important than the coalition. Consistent with this idea, Plavcan et al. [37] and Plavcan [50] found evidence that species with strong male-male or female-female coalitions have reduced canine size for their physical competition intensity. Increasing the importance of coalitions could therefore produce a primate with reduced dimorphism and high BS for its competition level, diminishing the pattern this study looked for [37, 50]. This study also did not look at any effects that female-female competition could have on the predicted relationship between dimorphism and BS [11, 37, 45, 50]. Female-female competition would decrease dimorphism if the competition caused selection for increased female canine size as in males and complicate a signal between dimorphism and BS. It is also possible that females could lower dimorphism by choosing subordinate males [51]. The possible effect of female-male competition was also ignored in this study [11, 50]. However, this type of competition can be important to some primate species, especially if it is an infanticidal species [see ref. in 52]. All of these possibilities would add noise to the data analyzed here and reduce the correlation between our indices of male-male physical competition and brain size.

Another confounding factor is the possibility that physical male-male competition is important to some species but has diminished or no importance in others. For example, singing has been demonstrated to be important to dominance within some primate species, mainly gibbons [53]. Recently, Dunham and Rudolf [54] showed that copulatory plugs are present in species that are size-monomorphic even in the face of male-biased operational sex ratios. However, it is very difficult to measure the importance of male-male competition within a particular species without knowing quantitatively the fitness gained from competing well, the influence of female choice [51], and effects on dimorphism from other socioecological factors (diet, group size, etc.).

Brain size can also be increased from selection pressures other than male-male competition. A primate might need increased spatial-temporal memory to find females, but does not have to compete for females once located. This scenario would produce a primate with increased BS without high levels of dimorphism. For example, these traits could be needed by male Gray mouse lemurs (*Microcebus murinus*) because of wide female spatial-temporal dispersion, over-lapping male ranges, and little evidence of mate guarding [34]. It has also been proposed that frugivorous species might need to generate spatial and temporal maps to locate ripe fruit at the appropriate time [18, 19]. This would be a cognitive intensive task and might lead to the evolution of larger brain size [18, 19]. Sociality (a.k.a. Machiavellian Intelligence) also has a demonstrated influence on the primate brain [21]. The selective pressures from task other than male-male competition are apparent and certainly influence brain size.

There is also some debate about the proper way to control for allometric trends between brain size and body size [e.g., 55, 56, 57]. Total body mass was the only variable available to control for observed allometric trend and so was used. It has also been pointed out that body mass is a not a very descriptive measure. Rather it is a composite measure of the various types of tissues of the body. We do not deny this, but in direct support of the use of body mass dimorphism as a measure of something biologically relevant, we would point out the many studies that directly tied this ratio to independent measures of competition intensity across all of the phylogenetic groups of primates [10, 11, 14, 53].

Additionally, the use of different measures of male-male competition complicates interpretation. Variations in the way primates' fight give different levels of importance to the measures (SSD & CSD) used within individual groups [58]. For example, canines have been suggested to play a more important role than body mass in the intraspecific competition of Colobines [58]. Also, size and canine dimorphism are each subject to different selection pressures and constraints [50]. There are also problems and constraints on the usefulness of the measures themselves that result from how they are calculated (see Smith [59] for comprehensive review). It is also possible that the indices of competition are serving as a proxy for some other measure that we did not analyze. These limitations need to be kept in mind when interpreting the results.

These results should be deemed preliminary. We concede that this analysis is only a beginning step and is not final. Therefore, results should be

viewed in a more qualitative light. A more complete analysis, including many more covariates, will be possible as taxon-wide data becomes available. The ideal data set would also include data on specific components of the brain, which would allow for more precise hypotheses. Most importantly, including most of the known covariates will allow investigators to assess the relative importance of social, ecological, and life-history factors.

All of the above suggestions of confounding factors illustrate the complicated nature of dimorphism [58]. Nevertheless, given all of the factors that the current study excluded, it is intriguing that significant relationships were found; especially strong, consistent ones such as observed for the all primates and Old World monkeys groups.

This study was performed to test the hypothesis that physical male-male competition is one of the factors that participated in the evolution of large relative brain size in primates. We found that across several primate groupings there are strong positive relationships between female brain size and male-male competition intensity. These results are consistent with the hypothesis. There was however, no consistent trend between male brain size and competition intensity. Taken with the results of Lindenfors et al. [26], we conclude that physical male-male competition intensity is influencing primate brain size, although at a relatively small level. We anticipate that physical male-male competition intensity is likely to be a general driver of brain size within some groups of animals. This study provides evidence that physical male-male competition should be considered when researching primate brain evolution.

Importantly, this conclusion is complimentary, not competitive, to other hypotheses. Future research should focus on expanding models to include male-male competition with other social/ecological factors to help determine the importance of male-male competition to the primate brain and within other taxa.

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Table 5.1. Best-fit Models of SSD and CSD *versus* male and female brain size for multiple phylogenetic subgroupings within primates.

	Female						
Phylo. Subgrouping		Variables	b	*p*-value	Model	λ value	Δ AIC
	All				λ	0.992	2.72
		Body Mass	**0.590**	**<0.001**			
		SSD	**0.327**	**<0.001**			
	Hap				λ	0.992	2.35
		Body Mass	**0.588**	**<0.001**			
		SSD	**0.306**	**<0.001**			
	Strep				BM	-	1.7
		Body Mass	**0.589**	**<0.001**			
		SSD	**0.752**	**0.0198**			
	NWM				BM	-	1.04
		Body Mass	**0.626**	**<0.001**			
		SSD	0.260	0.0765			
	OWM				λ	0.883	9.45
		Body Mass	**0.498**	**<0.001**			
		SSD	**0.308**	**<0.001**			
	GA				BM	-	1.55
		Body Mass	**0.495**	**<0.001**			
		SSD	**0.309**	**0.009**			

Table 5.1. Continued

Male Phylo. Subgrouping	Variates	b	*p*-value	Model	lambda	Δ AIC
All				λ	0.981	11.09
	Body Mass	**0.616**	**<0.001**			
	SSD	**-0.235**	**0.003**			
Hap				λ	0.987	3.93
	Body Mass	**0.593**	**<0.001**			
	SSD	**-0.210**	**0.012**			
Strep				λ	0.905	6.64
	Body Mass	**0.654**	**<0.001**			
	SSD	0.103	0.7793			
NWM				BM	-	0.01
	Body Mass	**0.662**	**<0.001**			
	SSD	**-0.405**	**0.011**			
OWM				λ	0.871	7.69
	Body Mass	**0.465**	**<0.001**			
	SSD	-0.089	0.374			
GA				BM	-	1.16
	Body Mass	**0.476**	**<0.001**			
	SSD	0.066	0.631			

Hap= Haplorhine, Strep= Strepsirrhine, NWM= New World Monkeys, OWM= Old World Monkeys, GA= Gibbons & Apes. SSD= Sexual Size Dimorphism, CSD= Canine Size Dimorphism. Bold values are staticially signigicant.